



UNIVERSITI PUTRA MALAYSIA

**GROWTH, MORPHOLOGICAL AND GENETIC VARIATION OF
AZADIRACHTA EXCELSA (JACK) JACOBS GENOTYPES**

HAZANDY ABDUL HAMID

FK 2001 24

**GROWTH, MORPHOLOGICAL AND GENETIC VARIATION OF
AZADIRACHTA EXCELSA (JACK) JACOBS GENOTYPES**

By

HAZANDY ABDUL HAMID

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master Science in the Faculty of Forestry
Universiti Putra Malaysia**

January 2001

*Specially dedicated
to my family:*

*My beloved mami and papa
Wife and son
Sisters (Haziana, Haslindaayu, Hasnorulhuda, Hazanarulfaeda)
Brothers in-law (Mohd. Zuhal, Azhar)*

*And close friends...
Thank you*

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

**GROWTH, MORPHOLOGICAL AND GENETIC VARIATION OF
AZADIRACHTA EXCELSA (JACK) JACOBS GENOTYPES**

By

HAZANDY ABDUL HAMID

January 2001

Chairperson : Associate Professor Nor Aini Ab Shukor, Ph.D.

Faculty : Forestry

A study was made on six seed sources of *Azadirachta excelsa* (Jack) Jacobs at ages one and two years at two different locations, i.e. Rantau Panjang (Selangor) and Merchang (Terengganu) to determine the patterns of inter- and intra-specific variation in growth, genetic and morphological characteristics of this species. Measurements of survival, height, basal diameter (BD) and diameter breast height (DBH) were made on all trees at ages one and two years after planting in the field. Five characteristics of leaf morphology, i.e. leaf shape, leaf margin, leaf base, leaf angle and internode were also analysed for seed source variation. In addition, 19 enzyme systems were used to determine the genetic variation among seed sources using isozyme analysis.

All the seed sources survived well during the first year with 91.25% in Rantau Panjang and 70.00% in Merchang but the survival reduced to 86.45% and 41.33% respectively during the second year. The mean height values ranged from 126.85 cm to 209.38 cm in Rantau Panjang and 49.76 cm to 72.19

cm in Merchang during the first year, and 212.83 cm to 367.07 cm in Rantau Panjang and 85.40 cm to 156.16 cm in Merchang during the second year. Whereas, the mean values of BD ranged from 2.04 cm to 3.40 cm in Rantau Panjang and 0.64 cm to 0.87 cm in Merchang during the first year, and 3.33 cm to 5.65 cm in Rantau Panjang and 0.97 cm to 1.54 cm in Merchang during the second year. Meanwhile, the mean values of DBH for the first and second years ranged from 0.85 cm to 2.23 cm and 1.94 cm to 3.98 cm in Rantau Panjang respectively while in Merchang, the mean values of DBH ranged from 0.53 to 0.93 for second year.

There was no significant difference observed for three leaf morphology characteristics. Significant differences were observed only for leaf angle and internode. The morphological similarities ranged from 0.7930 to 0.9952. Isozyme analyses on 19 enzyme systems produced means of expected heterozygosities of 0.0575 to 0.0983 and the percentage of proportion of polymorphic loci varied from 31.43% to 42.86%. The extent of genetic identities ranged from 0.7727 to 0.9999. Generally, all seed sources were found to produce lower values of genetic diversity. Founder effects and seed sources or land races of the species were believed to be possible factors for such condition. Cluster analyses of both morphological and genetic parameters showed small genetic distance between seed sources and revealed the close relatedness of the seed sources which might be related to geographical and historical factors.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

**VARIASI PERTUMBUHAN, MORFOLOGI DAN GENETIK BAGI GENOTIP-
GENOTIP *AZADIRACHTA EXCELSA* (JACK) JACOBS**

Oleh

HAZANDY ABDUL HAMID

Januari 2001

Pengerusi : Profesor Madya Nor Aini Ab Shukor, Ph.D.

Fakulti : Perhutanan

Satu kajian telah dijalankan ke atas enam sumber biji *Azadirachta excelsa* (Jack) Jacobs pada umur satu dan dua tahun di dua percubaan provenan iaitu Rantau Panjang (Selangor) dan Merchang (Terengganu) untuk menentukan corak variasi pertumbuhan, morfologi dan genetic bagi spesis ini. Pengukuran kemandirian, ketinggian, perepang pangkal pokok dan perepang pada paras dada telah dilakukan ke atas pokok selepas satu dan dua tahun ditanam di lapangan. Lima ciri morfologi daun seperti bentuk, tepi, pangkal, sudut daun dan antara ruas telah dianalisa untuk variasi morfologi. Sebanyak 19 sistem enzim telah digunakan untuk menentukan variasi genetik di antara sumber-sumber biji dengan menggunakan analisa isoenzim.

Kesemua sumber biji menunjukkan nilai kemandirian di antara 91.25% di Rantau Panjang dan 70.00% di Merchang pada tahun pertama. Nilai kemandirian didapati menurun pada tahun kedua kepada 86.45% dan 41.33%. Nilai purata ketinggian berjulat di antara 126.85 cm dan 209.38 cm di

Rantau Panjang sementara di Merchang ia berjulat di antara 49.76 cm dan 72.19 cm pada tahun pertama dan meningkat kepada 212.83 cm dan 367.07 cm serta 85.40 cm dan 156.16 cm pada tahun kedua. Sementara itu, nilai purata bagi perepang pangkal pokok berjulat dari 2.04 cm ke 3.04 cm di Rantau Panjang dan 0.64 cm ke 0.87 cm di Merchang semasa tahun pertama dan masing-masing meningkat kepada 3.33 cm ke 5.65 cm dan 0.97 cm ke 1.54 cm pada tahun kedua. Manakala, nilai purata perepang pada paras dada adalah berjulat di antara 0.85 cm dan 2.23 pada tahun pertama serta 1.94 cm dan 3.98 cm pada tahun kedua di Rantau Panjang. Nilai purata perepang pada paras dada di Merchang hanya diperolehi pada tahun kedua.

Perbezaan yang bermakna hanya diperolehi dalam pengukuran sudut daun dan antara ruas bagi penentuan variasi morfologi. Nilai persamaan morfologi yang dikaji berjulat di antara 0.7930 dan 0.9952. Analisa isoenzim ke atas 19 sistem enzim menunjukkan nilai heterozigositi jangkaan berjulat di antara 0.0575 dan 0.0983. Peratusan lokus polimorfik yang diperolehi berjulat daripada 31.43% kepada 42.86%. Persamaan genetik pula bernilai di antara 0.7727 dan 0.9999. Umumnya, kesemua sumber biji yang dikaji menunjukkan nilai variasi genetik yang rendah yang mungkin dipengaruhi oleh kesan-kesan 'founder' dan sumber asal populasi. Analisa perkumpulan bagi parameter morfologi dan genetik menunjukkan jarak genetik yang kecil dan persamaan yang tinggi yang mungkin boleh diinterpretasikan oleh faktor sejarah dan jarak geografi.

ACKNOWLEDGEMENTS

Alhamdulillah, Praise to be for Allah S.W.T. which enables me to come this far. I would like to express my deepest appreciation to my supervisor, Associate Professor Dr. Nor Aini Ab Shukor for her supervision, dedication and support throughout this study in UPM. I also wish to express my deepest gratitude and thanks to my supervisory committee, Associate Professor Dr. Kamis Awang and Dr. Ab Rasip Ab Ghani for their invaluable suggestions and comments. My appreciation to Dean Faculty of Forestry, Associate Professor Dr. Rusli Mohd. for allowing me to use the facilities in the faculty throughout the study and also to PASCA scheme for supporting my study. My special thanks are also extended to Mr. Abd. Latib Senin, Mr. Salim Ahmad, Mr. Razak Sulong and Mr. Zakaria Taha for their assistance and co-operation during sample and information collection for the project.

I would also like to thank my beloved parents, family, wife, Rahayu Rany and friends, Mr. Rusdi, John, Griffin, Kalang, Chus and Saidy, who have encouraged, supported and advised me during my study. To all those who know me, thanks very much.

I certify that an Examination Committee met on 22nd January 2001 to conduct the final examination of Hazandy Abdul Hamid on his Master of Science thesis entitled "Growth, Morphological and Genetic Variation of *Azadirachta excelsa* (Jack) Jacobs Genotypes" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

JAMALUDDIN BASHARUDDIN, Ph.D.

Faculty of Forestry
Universiti Putra Malaysia
(Chairman)

NOR AINI AB SHUKOR, Ph.D.


Associate Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

KAMIS AWANG, Ph.D.

Associate Professor / Dean of Graduate School
Universiti Putra Malaysia
(Member)

AB RASIP AB GHANI, Ph.D.


Research Officer
Forest Research Institute of Malaysia
Kepong
(Member)



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor / Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 13 MAR 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.



MOHD/HAZALI MOHAYIDIN, Ph.D.

Professor

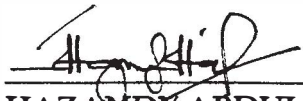
Deputy Dean of Graduate School

Universiti Putra Malaysia

Date: **12** APR 2001

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.


HAZANDY ABDUL HAMID

Date: 10.02.2001

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL SHEETS	viii
DECLARATION FORM	x
LISTS OF TABLES	xiv
LISTS OF FIGURES	xvi
LISTS OF PLATES	xvii
LISTS OF ABBREVIATIONS	xix
GLOSSARY	xxi
CHAPTER	
I GENERAL INTRODUCTION	1
II LITERATURE REVIEW	
<i>Azadirachta excelsa</i> (Jack) Jacobs	
General Background	10
Botany and Taxonomy of <i>A. excelsa</i>	11
Morphology	11
Distribution and Ecology	14
Growth and Development	15
Uses of <i>A. excelsa</i>	16
Importance of <i>A. excelsa</i> in Forest Plantation	18
Provenance Variations	20
Growth Performance	21
Morphological Variation	24
Genetic Variation	26
Electrophoresis	31
Isozyme	32
Association between Morphological and Genetic Variation	43
III MATERIALS AND METHODS	
Plant Materials	
Genotypes (Seed sources)	47
Field Establishment	48
Assessments and Analyses	53
Growth Variation (Performance)	53

	Morphological Traits	54
	Evaluation of Genetic Variation	55
	Isozyme Electrophoresis	
	Enzyme Extraction	56
	Gel and Electrode Buffer	56
	Mould and Starch Gel Preparation	57
	Electrophoretic Run	57
	Gel Slicing and Staining	59
	Data analysis	61
IV	RESULTS	
	Morphological Variation	
	Growth Variation (performance)	65
	Morphological Variability and Similarity	75
	Isozyme Analysis	
	Electrophoretic Patterns	80
	Intra-population Variation	98
	Genetic Structure	105
	Genetic Distance	107
	Correlation Analysis	
	Growth and Heterozygosity	110
	Genetic and Morphological (qualitative) Relationship	112
V	DISCUSSION	
	Growth Performance	113
	Intra- and Inter-population Variation	115
	Genetic Structure	122
	Cluster Analyses	125
	Correlation Analyses	127
VI	CONCLUSIONS AND RECOMMENDATIONS	
	Conclusions	130
	Recommendations	133
	REFERENCES	134
	APPENDIX	
A	Leaf Extraction Buffer	162
B	Buffer Systems	163

C	Enzyme Staining Recipes	164
D	Fixing solution	168
VITA		169

LIST OF TABLES

Table		Page
1.1	Forest cover area and rate of deforestation in the tropics by main ecological zone as estimated by Forest Research Assessment 1990.	5
2.1	Average growth of <i>A. excelsa</i> from age 1 to age 30 years in Nakhon Si Thammarat, Phatthalung, Trang and Songkla.	17
2.2	Genetic diversity measures for five seed sources of <i>A. excelsa</i>	40
3.1	Details of the six seed sources of <i>A. excelsa</i>	48
3.2	Details of the study sites.	49
3.3	Enzymes assayed, their abbreviations and Enzymes Commission (EC) designations.	60
4.1	Results of analysis of variance for growth parameters.	66
4.2	Site ranking based on overall performances of growth parameters.	67
4.3	Means of survival, height, BD and DBH for six seed sources of <i>A. excelsa</i> in Rantau Panjang and Merchang at one and two years respectively.	68
4.4	Leaf characteristics of six <i>A. excelsa</i> seed sources.	75
4.5	Analysis of variance for qualitative and quantitative traits of morphological variants.	77
4.6	Morphological identity (above diagonal) and morphological distance (below diagonal) based on the morphological characteristics.	79
4.7	Allele frequencies, observed (H_o) and expected heterozygosities (H_e) of six <i>A. excelsa</i> seed sources.	99

4.8	Summary of genetic diversity for six seed sources of <i>A. excelsa</i> using isozymes.	104
4.9	F-statistics and gene flow for all loci (Nei's, 1987).	106
4.10	Nei's (1973) statistics of genetic diversity for 25 polymorphic loci in six seed sources of <i>A. excelsa</i>	107
4.11	Nei's (1972) Coefficients of genetic identity (above diagonal) and genetic distance (below diagonal) among six seed sources of <i>Azadirachta excelsa</i>	109
5.1	The range and the mean of heterozygosity observed in some tree species.	116
5.2	The range and the mean of polymorphic loci observed in some forest trees.	118
5.3	The range and the mean number of allele per locus obtained from some forest trees.	119
5.7	Comparisons between levels and patterns of allozyme variation and other types of genetically controlled characteristics.	129

LIST OF FIGURES

Figure		Page
1.1	Forest areas by main regions in 1995.	4
3.1	Location of study sites and seed sources of <i>A. excelsa</i> .	50
3.2	Layout of provenance trial with Randomised Complete Block Design in Rantau Panjang Forest Reserve, Batu Arang, Selangor.	51
3.3	Layout of provenance trial with Randomised Complete Block Design in Merchang Forest Reserve, Merchang, Terengganu.	52
3.4	A 3-D section of the set up of the horizontal starch gel electrophoresis.	58
4.1	Dendrogram of six seed sources of <i>A. excelsa</i> based on morphological distances.	79
4.2	Banding patterns of nineteen enzymes examined in <i>A. excelsa</i>	81
4.3	Dendrogram of six seed sources of <i>A. excelsa</i> using Un-Weighted Pair Group Cluster Analysis Of Identity Coefficients (Nei's, 1972).	109
4.4	Relationships between means expected heterozygosities and overall means of growth parameters for first and second year.	111
4.5	Relationships between mean morphological distance and genetic distance, $r = 0.59$	112

LIST OF PLATES

Plate		Page
4.1	Two year performance of trees from Bukit Lagong seed source in Rantau Panjang.	71
4.2	A poor performance of trees from Pengkalan Arang seed source at two years in Rantau Panjang.	71
4.3	A good performance shown by trees from Narathiwat seed source at two years.	72
4.4	Manong seed source also performed well in Rantau Panjang.	72
4.5	Good growth shown by trees from Semengoh seed source at two years in Rantau Panjang.	73
4.6	A poor performance shown by trees from Pasir Mas seed source in Rantau Panjang.	73
4.7	Two year performance of <i>A. excelsa</i> in Merchang.	74
4.8	Exceptional height attained by a few trees in Merchang.	74
4.9	Leaf shape showing two patterns, i.e. lanceolate and ovate.	76
4.10	Leaf base showing two patterns, i.e. asymmetric and cuneate.	76
4.11	Leaf margin showing three patterns, i.e. toothed, entire and wavy.	77
4.12	The actual banding patterns observed for ACO enzyme.	81
4.13	An example of banding patterns observed for GPDH enzyme.	87

4.14	An example of banding patterns observed for MDH enzyme.	87
------	--	----

LIST OF ABBREVIATIONS

ACO	Aconitase
ADH	Alcohol dehydrogenase
ADK	Adenylate kinase
BD	Basal Diameter
cm	Centimetre
DBH	Diameter at breast height
EST	Esterase
FAO	Food and Agriculture Organisation
F_{IT}	Inbreeding coefficient of individuals in the total population
F_{IS}	Inbreeding coefficient of individuals in a sub population
F_{ST}	Inbreeding coefficient of subpopulations in a total population
GDH	Glutamate dehydrogenase
GDP	Gross Domestic Product
GLD	Glycerol dehydrogenase
GOT	Glutamate oxalate transaminase
GPDH	Glucose-6-phosphate dehydrogenase
I	Shannon's information index
IDH	Isocitrate dehydrogenase
LAP	Leucyl-aminopeptidase

m	Meter
mL	Millilitres
MDH	Malate dehydrogenase
ME	Malic enzyme
Nm	Gene flow
PER	Peroxidase
6PGD	6-Phosphogluconic dehydrogenase
PGI	Phosphoglucose isomerase
PGM	Phosphoglucomutase
SDH	Sorbitol dehydrogenase
ShDH	Shikimate dehydrogenase
SS	Sum of Square
TO	Tetrazolium oxidase

GLOSSARY

Adaptation	A change on the part of an individual or population resulting in better survival or growth.
Allele	One of a pair of genes or multiple of a gene. All alleles of a series occupy the same site or locus on a pair of homologous chromosomes.
Allozyme	Allelic forms of an enzyme that can be distinguished by electrophoresis
Amino groups	Group of form atoms, NH_2 forming part of a molecule.
Amino acid	Compounds containing one or more carboxyl and amino groups that polymerise.
Asexual reproductive	Reproduction without fertilization. It includes apomixis and various forms of vegetative reproduction.
Bisexual	Hermaphroditic, having both male and female reproductive organs. Usually used to indicate a departure from normality.
Breeding	The science of art of changing the genetic constitution of population of plants or animals.
Chromosome	Thread-like DNA molecule in cells that carry linearly-arranged genetic units.
Codominant alleles	Alleles of a given gene whose properties can be detected in a heterozygote.
Dimer	The complex of two polypeptides. These can be the same (in a homodimer) or different (in a heterodimer).
Diversity	The condition of being different exhibiting differences
Dioecious	Producing male and female flowers on different plants/trees.

Dominant	Description of an allele whose properties can be detected even in the presence of another allele.
Drift	Change in gene frequency and population characteristics due to chance rather than selection and usually most pronounced in small populations.
Electrophoresis	A process in which charged molecules migrate through an electric field.
Enzyme	Proteins that promote chemical processes of life without themselves being altered.
Ex situ	Out-of-place; material moved from its place of origin.
Evolution	Long-time changes in gene frequency and phenotypic characteristics of a population or group of populations.
Fixation	In population genetics, a condition in which all members of a population are homozygous for a given allele.
Gene	Basic unit of inheritance - a section of chromosome that codes for a genetic character.
Gene flow	The movement of genes through or between populations as the result of outcrossing and natural selection.
Genetic conservation	The conservation of genes or alleles for future use.
Genetic drift	A change in gene frequency that is a consequence of the continual random gain and loss of gametes and individuals in a population.
Genome	Total genetic content of an individual or species.
Genotype	The genetic constitution of an individual or group that may be either expressed or unexpressed, depending on the environmental effects of a given location
Hardy-Weinberg equilibrium	Stability in frequency of alleles and genotypes in a population generation after generation. A state of

equilibrium in a population's gene pool.

Heterozygosity	Proportion of heterozygous individuals in a population.
Heterozygous	Having different alleles at one or more loci when in a diploid or polyploid condition.
Homogenate	A finely divided and mixed tissue.
Homozygosity	The condition of having the same alleles at corresponding loci on homologous chromosomes.
Inbreeding	The intentional or unintentional breeding or crossing of individuals that are more closely related than their parents.
Isozyme	Different forms of the same enzyme - may be formed by different loci or different alleles at the same locus, in which case they can be termed "allozymes"
Loci	Plural of locus.
Locus	Position of gene in a chromosome.
Marker	An identifiable physical location on a chromosome whose inheritance can be monitored.
Mating System	Pattern by which gametes unite to form the next generation.
Monomer	A single molecular entity that may combine with others to form more complex structures.
Morphometric	The study of the physical shape and form.
Mutation	Change in genotype of an individual.
Monomorphic	Having a single form, lacking variation.
Outcrossing	Sexual reproduction between unrelated individuals or individuals of different genotypes, usually under natural conditions.

Phenotype	The sum total of the environmental and genetic (hereditary) influences on a tree; the visible characteristics of a plant.
Polymorphic	Occurrence of different forms in the same population or species.
Polymorphism	A detectable difference at a particular marker occurring among individuals.
Population	A group of organisms of the same species that occupy a particular geographic area or region. In general, individuals within a population interbreed with one another.
Provenance	The location of the source of plant material. For trees, an identifiable region in the natural habitat of a species from where the seed of the trees originally came.
Seed source	The location of the source of seed/plant material.
Selection	Any natural or artificial process that permits an increase in the proportion of certain genotypes or groups of genotypes in succeeding generations in relation to others
Self-fertilisation (selfing)	The natural or artificial process of placing pollen grains on a receptive stigma of the same individual.