



UNIVERSITI PUTRA MALAYSIA

**GENETIC DIVERSITY OF
ACACIA CRASSICARPA A. CUNN. EX BENTH.PLUS
TREES OF A PROVENANCE TRIAL IN SERDANG, MALAYSIA**

JOHN KEEN CHUBO

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**GENETIC DIVERSITY OF
ACACIA CRASSICARPA A. CUNN. EX BENTH.
PLUS TREES OF A PROVENANCE TRIAL IN SERDANG, MALAYSIA**

By

JOHN KEEN CHUBO

**Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the
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LIST OF ABBREVIATIONS

ACON	Aconitase
ADH	Alcohol dehydrogenase
bp	Base pair
χ^2	Chi-square
EST	Esterase
F Index	Fixation index
FIT	Inbreeding coefficient of individuals in the total population.
FIS	Inbreeding coefficient of individuals in a sub population.
FST	Inbreeding coefficient of subpopulations in a total population.
Nm	Gene flow
GD	Glucose dehydrogenase
GPI	Glucose phosphate isomerase
GOT	Glutamate oxaloacetate transaminase
G2DH	Glycerate dehydrogenase
GLYD	Glycerol dehydrogenase
IDH(NAD)	Isocitrate dehydrogenase (Nicotamine Adenine)
IDH (NADP)	Isocitrate dehydrogenase (Nicotamine Adenine Diphosphate)
Ind.	Indonesia
kb	Kilo base pair
LAP	Leucyl-aminopeptidase



MgCl ₂	Magnesium chloride
MDH	Malate dehydrogenase
ME	Malic enzyme
MD	Mannitol dehydrogenase
μL	Microlitres
mL	Millilitres
M	Molar
6PGD	6-Phosphogluconic dehydrogenase
pH	Negative logarithim of the hydrogen concentration
OPA	Operon primer set-A
OPB	Operon primer set-B
OPC	Operon primer set-C
OPG	Operon primer set-G
PER	Peroxidase
PGM	Phosphoglucomutase
PNG	Papua New Guinea
QLD	Queensland
RAPD	Random amplified polymorphic DNA
SDH	Shikimate dehydrogenase
SORDH	Sorbitol dehydrogenase
S.E.	Standard error
TO	Tetrazolium oxidase

GLOSSARY

Allele	One of two or more alternative forms of a gene, differing in DNA sequence and affecting the function in of a single gene product (RNA and/or protein). All alleles of a series occupy the same site or locus on a pair of homologous chromosomes.
Amplification	The production of many DNA copies from one master region of DNA.
Anneal	The spontaneous pairing of complementary DNA or RNA sequences by hydrogen bonding to form a double-stranded polynucleotide.
Apomixis	Asexual reproduction in plants in which the sexual organs or related structures are involved, but fertilisation does not occur. The resulting seed is vegetatively produced from an unfertilised egg or from somatic cells associated with the female parent.
Arbitrary primer	A short oligonucleotide primer used in certain PCR methods to initiate DNA synthesis at random locations on the target DNA.
Base	The chemical unit which characterises a nucleotide. In DNA the bases found are adenine, guanine, thymine and cytosine.
Base pair	Two nucleotide bases on different strands of a nucleic acid molecule that are held together by hydrogen bonds. Bases can pair in only one way - adenine with thymine and guanine with cytosine in DNA.
Chloroplast	Organelles found only in plants and photosynthetic proteins that absorb sunlight and use it to drive the synthesis of organic compounds from carbon dioxide and water.
Codominant alleles	Alleles of a given gene whose properties can be detected in a heterozygote.
Cytosolic fraction	The soluble fractions of cytoplasm, remaining after all particulates have been removed.
Denature	To provoke structural changes in a molecule which disrupt its biological activity. In DNA, it refers to the separation of the two component strands caused by the breaking of the hydrogen bonds. In proteins, it refers to disruptions in the

secondary and tertiary structure of the protein, destroying its activity.

Dimer	The complex of 2 polypeptides. These can be the same (in a homodimer) or different (in a heterodimer).
DNA	Deoxyribonucleic acid. The genetic material of most organisms. It is an organic acid and polymer consisting of four nitrogenous bases (adenine, cytosine, guanine and thymine) and a sugar-phosphate backbone. It usually exists as a double-stranded molecule in which the two antiparallel strands are held together by hydrogen bonding between the bases adenine-thymine and cytosine-guanine.
Dominant	Descriptive of an allele whose properties can be detected even in the presence of another allele.
Electrophoresis	A technique for separating molecules in a matrix (such as agarose or starch gels) according to their electrical charge and size.
Enzyme	A specialised protein catalyses biochemical reactions.
Ex situ	Plants which are planted outside its natural origin.
Fixation	In population genetics, a condition in which all members of a population are homozygous for a given allele.
Gene	The functional unit of heredity - a locus on a chromosome which encodes a specific functional product.
Gene flow	The movement of genes through or between population or species, the possession of a variety of genetic traits that frequently result in differing expressions in different individuals.
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.
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	The condition of having different alleles at corresponding loci on homologous chromosomes.
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.
Linkage	The physical association of genes on the same chromosomes.
Loci	Plural of locus (refer <i>locus</i>).
Locus	A specific site on a chromosome, usually of a gene or other marker.
Marker	An identifiable physical location on a chromosome whose inheritance can be monitored.
Mitochondria	Organelles in eukaryotic cells that serve as sites of cellular respiration.
Monomer	A single molecular entity that may combine with others to form more complex structures.
Monomorphic	The situations in which all the individuals in a population are the same genetic type or have the same allele.
Morphometric	The study of the physical shape and form.
Mutation	Change in genotype of an individual.
Organelles	Structures within eukaryotic cell in which certain functions and processes are localised.
Outcrossing	The breeding of unrelated plants or plants of different genotypes, usually under natural conditions.
Panmictic	A population in which mating occurs at random.
PCR	Polymerase Chain Reaction. A method for amplifying a DNA sequence in large amounts using a heat-stable polymerase and suitable primers to direct the amplification of the desired region of DNA.
Phenotype	The observable characteristics of an organism due to the interaction between the genotype and the environment.

Polymerase	General term for enzymes which carry out the synthesis of nucleic acid using a pre-existing nucleic acid template and the appropriate nucleotides.
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.
Primer	A short DNA fragment annealed to a single-stranded DNA, to which further nucleotides can be added by DNA polymerase.
Protein	A polymer of amino acids joined by peptide bonds, which may be comprised of two or more polypeptide chains.
Provenance	Origin of source; for trees, an identifiable region in the natural habitat of a species from where the seed of the trees originally came.
Putative	Commonly accepted, supposed or assumed to exist or to have existed.
Random mating	Mating between individuals where the choice of a partner is not influenced by the genotypes (with respect to specific genes under study).
RAPD	Random Amplified Polymorphic DNA. A widely used technique for amplifying anonymous stretches of DNA using PCR with arbitrary primers.
Self-Fertilisation (Selfing)	The natural or artificial process of placing pollen grains on a receptive stigma of the same individual.

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**GENETIC DIVERSITY OF *ACACIA CRASSICARPA* A. CUNN. EX
BENTH. PLUS TREES OF A PROVENANCE TRIAL IN
SERDANG, MALAYSIA**

By

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July 1999

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

Faculty : Forestry

In the selection of the best species for forest plantation, few criterias have to be considered including morphological and genetic diversity. Thus, the objective of this study was to investigate the diversity of *Acacia crassicarpa* plus trees using morphological and genetic markers. The genetic structure and mating system of the species were also studied.

Plus trees were selected from a provenance trial in Universiti Putra Malaysia (UPM), Serdang based on 8 qualitative and 2 quantitative parameters from 2 regions i.e. Queensland (8 plus trees) and Papua New Guinea (23 plus trees). Leaf samples were collected from these trees and were analysed using 20 isozyme and 15 RAPD markers.



The morphological study observed higher variation and better growth performance of trees from Papua New Guinea. However, trees from Queensland have higher retention towards strong wind contradictory to trees from Papua New Guinea. The isozyme analysis observed 36 loci with 24 loci being polymorphic. The mean expected heterozygosities were 0.2316 and 0.2675 for Queensland and Papua New Guinea respectively. The proportions of polymorphic loci for both regions were found to be similar. In the RAPD analysis, a total of 87 loci were scored ranging from 0.10 kb to more than 2.10 kb. Generally, provenances from Papua New Guinea were found to produce higher polymorphism levels as compared to the Queensland provenances. Life history and the ecological characteristics of the species were believed to be the possible reasons for such conditions. Cluster analyses produced three different dendrogram patterns with a tendency of similarity to a certain. The effect of different approaches was suggested to have caused these differences. Relationships of clusters according to number of parent trees, altitudes and longitudes were also observed.

The species was found to be highly outcrossing with rates ranging from 0.69 and 0.94. Genetic differentiation in the species observed 60 to 70% of the total diversity to be within provenances. Factors such as reproductive biology, seed dispersal, history and gene flow were suggested to be some of the possible causes for such phenomena.



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

**KEPELBAGAIAN GENETIK POKOK PLUS *ACACIA CRASSICARPA*
A. CUNN. EX BENTH. DARI KAWASAN PERCUBAAN PROVENAN DI
SERDANG, MALAYSIA**

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Dalam pemilihan spesis yang paling sesuai untuk hutan ladang, beberapa kriteria hendaklah diambilkira termasuk faktor morfologi dan kepelbagaian genetik. Maka, objektif kajian ini adalah mengkaji kepelbagaian pokok plus *Acacia crassicarpa* menggunakan kaedah morfologi dan penanda genetik. Struktur genetik dan sistem persenyawaan spesis tersebut juga turut dikaji.

Pokok-pokok plus dipilih dari kawasan percubaan provenan di Universiti Putra Malaysia (UPM), Serdang berdasarkan 8 parameter kualitatif dan 2 parameter kuantitatif dari 2 daerah iaitu Queensland (8 pokok plus) dan Papua New Guinea (23 pokok plus). Sampel daun yang dikutip dari pokok-pokok ini dianalisa menggunakan 20 penanda isoenzim and 15 penanda RAPD.



Kajian morfologi menunjukkan pokok-pokok dari Papua New Guinea mempunyai variasi yang lebih tinggi dan pertumbuhan yang lebih baik. Sungguhpun begitu, pokok-pokok dari Queensland mempunyai kadar rintangan angin yang lebih baik daripada Papua New Guinea. Analisa isoenzim menunjukkan kewujudan 36 lokus dengan 24 lokus polimorfik. Nilai heterozigositi jangkaan adalah 0.2316 untuk Queensland dan 0.2675 untuk Papua New Guinea. Peratusan lokus polimorfik untuk kedua-dua daerah adalah serupa. Dalam analisa RAPD, 87 lokus telah diambil kira dan berjulat dari 0.10 kb ke lebih dari 2.10 kb. Umumnya, provenan-provenan dari Papua New Guinea mempunyai nilai polimorfisma yang lebih tinggi daripada Queensland. Kejadian ini dipercayai berhubungkait dengan sejarah kehidupan dan ciri-ciri ekologi spesis tersebut. Analisa perkumpulan menghasilkan tiga dendrogram yang berlainan sungguhpun terdapat sedikit persamaan di antaranya. Dalam hal ini, penggunaan kaedah-kaedah yang berlainan dipercayai telah memberikan kesan-kesan yang berlainan. Hubungan perkumpulan berdasarkan jumlah pokok ibu, altitud dan longitud juga turut dibincangkan.

Kadar persenyawaan silang didapati tinggi dalam spesis ini dan berjulat dari 0.69 ke 0.94. Perbezaan genetik mendapati 60 hingga 70% daripada keseluruhan kepelbagaian spesis ini terkandung di peringkat provenan. Faktor-faktor seperti biologi pembiakan, penyebaran biji benih, sejarah dan hanyutan gen adalah punca yang mungkin untuk menerangkan fenomena ini.

CHAPTER I

GENERAL INTRODUCTION

The world's forest has been reported to decline at an alarming rate. The area of the world's forests, including natural and plantation forests, was estimated to be 3,454 million hectares in 1995, or about one-fourth of the land area of the earth. Between 1990 and 1995, FAO (1997) estimated a net loss on 56.3 million hectares of forests worldwide. This represented a decrease of 65.1 million hectares in developing countries and an increase on 8.8 million hectares in developed countries. The area under natural forest in the developing countries particularly in Asia / Oceania was estimated to reduce by nearly 4 million hectares annually between 1980 and 1995 (Asian Timber, February 1998). In Malaysia alone, a decrease of about 2 million hectares between 1990 and 1995 has been reported by FAO (1997).

The global demand for all forest products will continue to rise. According to FAO, through its "most probable" growth scenario at 1% per year, demand is expected to reach 3.9 million m³ in 2010, of which half would be fuelwood, which is used largely in the developing countries (Asian Timber, January 1998). FAO has also projected a shortage of industrial roundwood of about 110 million m³ by the year 2010, with the consumption expected to reach 2.28 billion m³ compared to a



production estimated at 2.17 billion m³ (Asian Timber, February 1998). In Malaysia, the annual log production in the Seventh Malaysia Plan was expected to be 28.3 million m³ with 7.4, 5.4 and 15.5 million m³, for Peninsular Malaysia, Sabah and Sarawak respectively, which represented a decrease of 17% compared to the average of 34 million m³ per year under the Sixth Malaysia Plan (Thai, 1995). The local supply of log up to the year 2010 has been projected to be about 13.7 million m³ (Abdul Razak, 1997), implying a reduction in log production of more than 17%.

On the other hand, the total annual installed processing capacities of mills in Peninsular Malaysia, Sabah and Sarawak are 13.2, 7.45 and 6.0 million m³ respectively (Mahmud, 1997). One key determinant in future development of the downstream wood-based industry is the sustainable supply of raw materials, particularly logs. Thus, access to a reliable supply of trees for raw materials will continue to remain as an important determining indicator in this sector. In order to overcome deficit of raw materials as well as to ensure sustainable supply of timber for wood-based downstream industry, extensive tree planting is urgently needed (Mahmud, 1997).

Presently, the rehabilitation of deforested area has been practised in certain parts of South East Asia through the establishment of forest plantations. To date there are about 80 to 100 million hectares of forest plantations in the developed countries while in the developing countries the total area of forest plantations have increased from 40 million hectares in 1980 to about 81 million hectares in 1990 (Asian Timber, February 1998).

In Malaysia, forest plantation has long been recognised as an essential part of a strategic development plan for the sustainable management of forest resources (Thai, 1994). Fast growing species of both indigenous dipterocarps as well as exotic species such as *Gmelina arborea*, *Paraserianthes falcataria* have been identified as suitable species for planting particularly for the production of general utility timbers (Thai, 1994). More recently, Krishnapillay *et al.* (1997) revealed that *Tectona grandis* and *Azadirachta excelsa* are very viable as plantation crops on a 15- year rotation. Another genus that has drawn much attention as a plantation species is the fast growing tropical *Acacia*. Several species of this genus have proven themselves as potential multiple plantation species (Turnbull, 1986) in many developing countries which cover a range of climatic zones such as the humid tropics, cool tropical highlands and arid or semi-arid regions (Logan, 1987).

The success of sustainable forest management will also require the understanding and effective management of other aspects such as ecology and genetics besides the biology aspect. Genetic studies in particular, have been used to identify superior species populations or provenance and played an important role in the subsequent selection and breeding of the most desirable individuals within the populations. Moreover, the conservation of genetic diversity requires knowledge about the distribution and abundance of species, key mutualistic interactions among organisms, amount and patterns of genetic diversity, and progress in technology of long-term storage of propagules. Data on the levels and spatial distribution of genetic variation are of crucial importance to several aspects of tropical biology (Hamrick and Loveless, 1986). In addition, Bawa (1976) also stressed on the

importance of genetic information in tropical tree breeding programmes. Genetic variation found between and within species serves as an important buffer to the temporal and spatial variation of potential stress factors (Bergmann *et al.*, 1989) and also in the building blocks for human use in selection and breeding for adaptability to a range of environments and end uses. Thus, this will include the establishment, management and preservation of the maximum amount of genetic variation within species (Soule, 1980; Whitmore, 1980).

Isozyme electrophoresis using starch gel has been extensively used over the past several decades in investigations of the genetics of a large number of organisms from fruit flies and human to crop plants. In terms of forest species, isozyme analysis in particular genetic polymorphism has been reported in temperate trees. The species studied include those on *Pseudotsuga menziesii* (Yeh and O'Malley, 1980; Mejnartowicz and Lewandowski, 1994; Prat and Arnal, 1994), *Pinus strobus* (Beaulieu and Simon, 1994), *Pinus nigra* (Aguinagalde and Bueno, 1994) and *Chamaecyparis lawsoniana* (Millar and Marshall, 1991). Similar investigations have also been done on tropical trees such as those on genetic diversity and breeding systems of *Acacia*. These reports encompass those on *Acacia auriculiformis* (Moran *et al.*, 1989a, Wickneswari and Norwati, 1993), *Acacia crassicarpa* (Moran *et al.*, 1989a), *Acacia mangium* (Moran *et al.*, 1989b) and *Acacia melanoxylon* (Playford *et al.*, 1991). Other tropical species included *Pterocarpus macrocarpus* (Liengsiri *et al.*, 1994), *Hevea brasiliensis* (de Paiva *et al.*, 1994a and 1994b), *Tectona grandis* (Kertadikara and Prat, 1995) as well as *Eucalyptus urophylla* and *Eucalyptus grandis* (Martins-Corder and Lopes, 1997).