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GENETIC DIFFERENTIATION OF MALAYSIAN OAKS BY MICROSATELLITE MARKERS

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BY

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A combination of nuclear and chloroplast microsatellite DNA have been used to investigate the levels and pattern of variability in Malaysian oaks. This study focussed on *Quercus* sp., which is the largest and the most widely distributed genus in the family Fagaceae. In the nuclear microsatellite study, four sets of microsatellite primers developed for *Quercus* sp. (Dow *et al.*, 1995; Steinkellner *et al.*, 1997a; Isagi and Suhandono, 1997; Kampfer *et al.*, 1998) were tested. Based on the analysis, more than 35% of the 61 primers tested resulted in interpretable amplification products. Twenty microsatellite primers were used to estimate the genetic diversity among distributions of *Quercus* sp. These selected primers were also used in three other genera from the same family namely, *Lithocarpus*, *Castanopsis* and *Triganobalanus*.

The results showed that these microsatellite loci are conserved across different genera. Four primers, QpZAG9, QrZAG20, QrZAG31 and QrZAG108 gave interpretable PCR products for all the samples studied from the four genera. None of the microsatellite loci is monomorphic in all the species studied. The number of alleles per microsatellite locus varied from 2 to 20. On the average, 11.85 alleles per locus were

observed. The mean value of gene diversity ranged from 0.0141 at locus *QM50-3M* to 0.6494 at locus *QpZAG1/5* with a mean of 0.3162. The highest mean gene diversity (H_o) for all loci was 0.4290, which was observed in *Q. lineata* whereas the lowest H_o was found in *Castanopsis* sp. The genetic differentiation among the species was estimated as F_{st} = 0.6705. Three dendrograms based on Nei's genetic relationship (1978) clustered by the UPGMA method were constructed. The first dendrogram containing four different genera showed that *Lithocarpus* and *Castanopsis* are clustered in one group while *Triganobalanus* is clustered away from *Quercus*, *Lithocarpus* and *Castanopsis*. The second dendrogram showed that the main cluster is subdivided into two major subclusters. The Peninsula species was in one group whereas the Sarawak species formed another subcluster except for *Q. subsericea* from Banjaran Lumut. On the other hand, the third dendrogram which clustered individuals of all the species studied showed that all individuals from the same species is clustered together in the same group except *for Q. gemelliflora.* One unknown individual collected from the Kelabit Highlands is clustered together with *Q. sumatrana*. The estimates of genetic similarities based on microsatellite markers ranged from 0.0844 to 0.8590 among the different species.

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PERBEZAAN GENETIK DALAM OAK MALAYSIA DIUJI DENGAN PENANDA MIKROSATELIT.

PUNG CHAI CHIN
January 2001

Pengerusi : Professor Dr. Tan Soon Guan

Fakulti : Sains and Pengajian Alam Sekitar

Satu gabungan kajian mikrosatelit nukleus dan kloroplas DNA digunakan untuk mengkaji paras dan corak variabiliti bagi oak Malaysia. Kajian ini memokus kepada *Quercus* sp. di mana ia merupakan genera yang terbesar dan terluas dalam famili Fagaceae. Dalam kajian mikrosatelit nukleus, sebanyak empat set primer yang direka untuk *Quercus* sp. (Dow *et al.*, 1995; ; Isagi and Suhandono, 1997; Steinkellner *et al.*, 1997a dan Kampfer *et al.*, 1998) diuji. Daripada analisis, lebih daripada 35% dari 61 primer yang diuji memberi produk amplifikasi yang boleh diinterpretasikan. Dua puluh primer digunakan bagi menjangka diversiti genetik untuk *Quercus* sp. Primer yang dipilih ini juga diuji dalam tiga genera dari famili yang sama iaitu *Lithocarpus*, *Castanopsis* dan *Triganobalanus*.

Keputusan menunjukkan lokus mikrosatelit adalah terpelihara antara genera yang berlainan. Empat primer iaitu QpZAG9, QrZAG20, QrZAG31 dan QrZAG108 memberi produk PCR yang boleh diintepretasikan bagi semua sampel yang diuji berasal dari empat genera. Tiada lokus mikrosatelit yang monomorfik dalam semua sampel yang diuji. Nombor alel per penanda mikrosatelit adalah berlainan dari 2 hingga 20.

Secara puratanya, 11.85 alel diperhatikan. Purata diversiti gen pula berjulat dari 0.0141 pada lokus *QM50-3M* kepada 0.6494 pada lokus *QpZAG1/5* dengan purata 0.3162. Purata diversiti gen yang tertinggi ($\bar{H}_o$) dalam semua lokus ialah 0.4290, iaitu diperhatikan dalam *Q. lineata* manakala $\bar{H}_o$ yang terendah diperhatikan pada *Castanopsis* sp. Perbezaan genetik antara spesis dijangka dengan F_{st} = 0.6705. Tiga dendrogram berdasarkan perkaitan genetik Nei's (1978) dikelompok berdasar cara UPGMA dikendalikan. Dendrogram yang pertama yang berdasarkan empat genera yang berlainan menunjukkan bahawa *Lithocarpus* dan *Castanopsis* dikelompokkan dalam satu kumpulan manakala *Triganobalanus* dikelompokkan jauh daripada *Quercus*, *Lithocarpus* dan *Castanopsis*. Dendrogram kedua menunjukkan kelompok utama di bahagikan kepada dua kelompok utama. Spesis Semenanjung dalam satu kumpulan manakala spesis Sarawak dalam satu kumpulan yang lain kecuali *Q. subsericea* dari Banjaran Lumut. Dalam pada itu, dendrogram yang ketiga yang mengelompokkan semua individu sampel dari semua spesis menunjukkan semua individu yang berasal dari spesis yang sama berada dalam satu kumpulan yang sama kecuali *Q. gemelliflora*. Satu individu yang tidak dikenali yang telah dikumpul dari Kelabit Highland adalah dikelompokkan bersama *Q. sumatrana*. Similariti genetik yang dijangka berdasarkan penanda mikrosatelit pada puratanya berjulat dari 0.0844 ke 0.8590 antara spesis yang berlainan.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

1X	One time
A	Adenosine
bp	Base pair
C	Cytosine
cpSSR	Chloroplast Simple Sequence Repeat
D	Genetic Distances
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
EDTA	Ethylenediaminetracetic acid
G	Guanine
nSSR	Nuclear Simple Sequence Repeat
PCR	Polymerase Chain Reaction
PHYLIP	Phylogeny Inference Package
RFLP	Restriction Fragment Length Polymorphism
Sp	Species
SSR	Simple Sequence Repeat
T	Thymine
TBE	Tris borate EDTA
UV	Ultra violet
VNTR	Variable Number of Tandem Repeat

CHAPTER 1

INTRODUCTION

The occurrence of oak tree in Malaysia is often surprising to most people. It is widely distributed throughout Peninsular Malaysia, Sabah and Sarawak. In terms of classification and taxonomy, Malaysian oaks were well documented by Soepadmo (1966, 1968 and 1972), Keng (1969), Corner (1972), Ridley (1967) and Soepadmo *et al.* (2000) for Sabah and Sarawak *Quercus*.

Oak is a common name for trees of the genus *Quercus* in the European and Asian region. Oak also refers to the genus *Lithocarpus* in the Malesia region. It had been reported that locally *Quercus* sp. and *Lithocarpus* sp. were known as *Castanopsis*, another genus from the Fagaceae family. On the other hand, some *Quercus* sp. was earlier identified as *Lithocarpus* sp. (Corner, 1972). In fact, according to Soepadmo, identification based on morphological evidences suggest they are actually two different genera.

Oak wood is less famous for timber products and is only used in medium to heavy construction. However, the beautiful wood ray of the species is well known as a source of timber for the furniture and flooring industries. Some of the species have been tried for use in the cultivation of mushroom in Borneo. It has proven useful for controlling erosion when planted on steep slopes in mountainous regions (Sunarno *et al.*, 1995).

Studies of oaks in Malaysia were limited to taxonomy. Earlier investigations of relationships among Malaysian oak accessions were based on morphological characters. *Quercus* sp. and *Lithocarpus* sp. which occurred throughout Malaysia are normally distinguished using leaf and acorn morphology characters either for species or genus level identification, but there is no truly diagnostic character that can assign an individual tree or population to one or the other species with certainty.

Furthermore, morphological evidence should be avoided since they may not be reliable measure of genetic difference because of the influence of the environment on gene expression. However, the analysis of plant DNA allows the direct assessment of variation in the genotype. Direct diagnostic markers allow the immediate detection of alterations without uncovering the effects of the alteration.

At present, development of molecular markers provides additional information on plants. DNA data also provide superior information, and with the advent of the PCR method, it may eventually become the predominant class of data. Recently, microsatellite DNA has been proven to be very useful for the purpose of studying genetic diversity in forest tree species (Lefort *et. al.* 1999a, Ujino *et. al.* 1998, Dow and Ashley 1998). Microsatellites are stretches of DNA consisting of di-, tri-, or tetra-nucleotide repeats such as $(AT)_n$ or $(GT)_n$ that frequently extend up to 100 times. Polymorphisms in microsatellites result from differences in the number of these repeat units. They are highly variable and co-dominantly inherited and can be used in genetic diversity, ecological and evolutionary studies.

Microsatellite primers are usually confined to a single taxon from which the primers were developed. However, some examples have been described in which microsatellite loci are conserved in other closely related species (Ujino *et al.*, 1998, Echt *et al.*, 1999 & Lefort *et al.*, 1999), which have allowed the analysis of genetic diversity to be carried out in these related species. Steinkellner *et al.* (1997a) have described the conservation of microsatellite loci between *Quercus* species. Their results showed that microsatellites isolated from *Quercus petraea* are conserved in other *Quercus* species and even in other species within Fagaceae.

With the establishment of these nuclear microsatellite primers (Steinkellner *et al.*, 1997a; Isagi and Suhandono, 1997; Kampfer *et al.*, 1998 and Dow *et al.*, 1995) for *Quercus* sp., this study was focused on diversity and genetic differentiation within the *Quercus* sp. that occurred throughout Malaysia. The selected primers were also tested in other three genera namely *Lithocarpus* sp., *Castanopsis* sp. and *Triganobalanus* sp. which are from the same family, Fagaceae.

Microsatellites are not limited to the nuclear genome. They are also found in the chloroplast genome. The developers of chloroplast microsatellite (cpSSR) primers in dicotyledonous angiosperms (Weising and Gardner, 1999) suggested that these universal primers may serve as general tools to study chloroplast variation in angiosperms. Thus, this study also tested the capability of cpSSR primers for amplifying the Malaysian oak genome. The extend of cross-species amplification appears to be correlated with taxonomic distance. Therefore, microsatellites are able be used for phylogenetic studies through the construction of informative dendrograms based on the alleleic frequencies of microsatellite loci.

The objectives of this study were:

1. To develop microsatellite markers that could distinguish diagnostically between species Malaysian oaks.

2. To develop methodologies for typing microsatellites as genetic markers in Malaysian oaks.

3. To examine whether primer pairs designed to amplify microsatellite loci in other oak species could be used to amplify marker loci in Malaysian oaks.

4. To examine whether primer pairs designed to amplify chloroplast microsatellite loci in angiosperms could be used to amplify marker loci in Malaysian oaks.

5. To determine genetic relationships among *Quercus* sp., *Lithocarpus* sp., *Castanopsis* sp. and *Triganobalanus* by constructing a dendrogram.

6. To clarify the exact identity of the taxa of Malaysian oaks by determining their genetic distances and through constructing a dendrogram.

7. To investigate the population structure of each species by estimating the genetic diversity at the microsatellite loci.

8. To examine whether microsatellite markers are suitable for taxonomic studies by determining the relationships between the species studied.