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RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS OF FOUR BREEDS OF SHEEP IN MALAYSIA

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RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS OF FOUR BREEDS OF SHEEP IN MALAYSIA

By

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
BB	Barbados Blackbelly
Bp	Base pair
DMX	Dorset-Malin cross
M	Malin
mtDNA	Mitochondrial DNA
PCR	Polymerase Chain Reaction
PHYLIP	Phylogeny Inference Package
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SI	Santa Ines
VNTR	Variable number of tandem repeats

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GENETIC EVALUATION OF SOME BREEDS OF SHEEP IN MALAYSIA

By

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Chairman: Dr. Jothi M. Panandam

Faculty: Veterinary Medicine and Animal Science

The genetic variability and population structure of four populations of sheep breeds namely, Santa Ines, Barbados Blackbelly, Malin and the Dorset-Malin cross, was investigated using the Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). Eight 10-*mer* oligonucleotide primers of arbitrary sequence (OPD-03, OPD-09, OPD-11, OPD-12, BRL-02, BRL-03, BRL-05, BRL-06) were used to amplify discrete regions of the sheep genome from 30 individuals per breed. The amplified product was electrophoresed using agarose gels to reveal banding patterns that could differentiate between individuals. These primers revealed a total of 65 DNA markers with a range in size of 228-1,871 bp. Of these markers, 38 or 58.5% were polymorphic while 27 or 42.5% were monomorphic. Primer OPD-09 amplified seven bands but all bands were 100% monomorphic in the four breeds. Two markers, BRL03.1 and BRL03.5 were found to be common and exclusive for the wool sheep breeds. Genetic distance was calculated using both the Dice's and Jaccard's similarity indices. High genetic values

were seen within the Santa Ines breed (Dice: 0.0930; Jaccard: 0.1682) while the least variable population was the Dorset-Malin cross (Dice: 0.00693; Jaccard: 0.1280). Genetic distance values for Barbados Blackbelly and Malin was calculated as 0.0748 (Dice)/0.1378 (Jaccard) and 0.0813 (Dice)/0.0813 (Jaccard) respectively. The neighbour-joining and the UPGMA trees based on the Dice's and Jaccard's similarity indices showed little or no consensus on the clustering of the individuals in each population.

Genetic distance between populations ranged from 0.1040 to 0.1357 based on the Dice's algorithm while the distance based on the Jaccard's algorithm ranged from 0.1875 to 0.2381. Greatest similarity was observed between Santa Ines and Barbados Blackbelly breeds (Dice: 89.6%; Jaccard: 81.2%) while the Santa Ines and the Dorset-Malin cross were the least similar (Dice: 86.4%; Jaccard: 76.2%). The neighbour-joining and UPGMA analysis showed the Santa Ines and Barbados Blackbelly to be closely linked, while the hair sheep breeds were closely related to the Malin. The Dorset-Malin cross was genetically furthest away from the rest of the breeds. Consensus tree produced after 100 bootstrapping show a confidence level of 78% between the Santa Ines and Barbados Blackbelly branch and a 61% confidence level between the hair sheep breeds and the Malin.

Total number of phenotypes per primer ranged from 1 (OPD-09) to 62 (BRL-06). The Santa Ines population had a total of 61 phenotypes, which was the largest, followed by Barbados Blackbelly (56), Malin (54) and Dorset-Malin (43). All primers (except OPD-09) had phenotypes common either to the hair sheep or wool sheep breeds.

The analysis of molecular variance showed that four out of the seven polymorphic primers (OPD-11, OPD-12, BRL-03, and BRL-06) were able to detect variance among fleece type, among populations and among individuals in a population. Primers OPD-03 and BRL-02 were unable to detect significant variance among the hair and wool breeds, while primer BRL-05 was unable to detect significant variance among the populations. Primer OPD-11 detected the highest variance among individuals (91.08%) while primer BRL-02 detected the lowest variance among individuals (8.46%). Overall data showed 74% variance among individuals within population, 20% variance among populations and 4% variance among fleece types.

The genetic variability and structure of the four populations of sheep were identified through the RAPD-PCR assay. Such information is necessary in order to devise breeding or crossbreeding programs for the development of the sheep industry.

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

PENILAIAN GENETIK BEBERAPA BAKA BEBIRI DI MALAYSIA

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Variasi genetik dan struktur populasi empat baka bebiri iaitu Santa Ines, Barbados Blackbelly, Malin dan kacukan Dorset-Malin diselidik dengan kaedah *Random Amplified Polymorphic DNA-Polymerase Chain Reaction* (RAPD-PCR). Lapan primer oligonukleotid pendek 10-*mer* dengan jujukan rawak (OPD-03, OPD-09, OPD-11, OPD-12, BRL-02, BRL-03, BRL-05 dan BRL-06) digunakan untuk mengamplifikasi kawasan-kawasan diskrit pada genom bebiri daripada 30 ekor per baka. Produk-produk yang telah diamplikasikan seterusnya dielektroforesis menggunakan gel agarose untuk melihat corak jalur-jalur yang dapat membezakan antara individu-individu. Primer-primer ini telah menunjukkan sejumlah 65 penanda DNA dengan saiz berjulat antara 228-1,871 bp. Dari jumlah ini, 38 atau 58.5% penanda ini adalah polimorfik manakala 27 atau 42.5% monomorfik. Primer OPD-09 menunjukkan tujuh jalur tetapi kesemuanya adalah 100% monomorfik bagi keempat-empat baka berbiri tersebut. Didapati dua penanda, BRL03.1 dan BRL03.5 hanya terdapat pada bebiri berbulu.

Jarak genetik dikira menggunakan indek kesamaan Dice serta Jaccard. Nilai genetik yang tinggi dilihat pada baka Santa Ines (Dice: 0.0930; Jaccard: 0.1682) sementara populasi yang menunjukkan variasi paling sedikit ialah baka kacukan Dorset-Malin (Dice: 0.00693; Jaccard:0.1280). Nilai jarak genetik bagi Barbados Blackbelly dan Malin didapati masing-masing ialah 0.0748 (Dice)/0.1378 (Jaccard) and 0.0813 (Dice)/0.0813 (Jaccard). Pokok-pokok *neighbour-joining* dan UPGMA berdasarkan pada indeks-indeks kesamaan Dice dan Jaccard menunjukkan sidikit ataupun tiada konsensi keatas kelompok individu-individu dalam setiap populasi.

Didapati julat jarak genetik antara populasi berdasarkan pada algoritma Dice ialah antara 0.1040 hingga 0.1357, manakala julat jarak berdasarkan pada algoritma Jaccard ialah 0.1875 hingga 0.2381. Adalah diperhatikan bahawa persamaan yang paling tinggi ialah antara baka Santa Ines dan Barbados Blackbelly (Dice: 89.6%; Jaccard: 81.2%) sementara baka Santa Ines dan kacukan Dorset-Malin mempunyai persamaan yang paling kurang (Dice: 86.4%; Jaccard: 76.2%). Analisa *neighbour-joining* dan UPGMA menunjukkan Santa Ines dan Barbados Blackbelly mempunyai pertalian yang rapat manakala baka-baka bebiri rambut mempunyai kaitan yang rapat dengan Malin. Kacukan Dorset-Malin didapati berada jauh sekali dari segi genetik daripada baka-baka beberi yang lain. Pokok konsensi selepas 100 kali *bootstrapping* menunjukkan nilai tahap keyakinan 78% antara cabang Santa Ines dan Barbados Blackbelly sementara nilai tahap keyakinan antara baka bebiri rambut dan Malin ialah 61%.

Jumlah keseluruhan fenotip per primer berjulat antara 1 (OPD-09) hingga 62 (BRL-06). Populasi Santa Ines menunjukkan sejumlah 61 jenis fenotip, iaitu jumlah yang terbesar, diikuti oleh Barbados Blackbelly (56), Malin (54) dan Dorset-Malin (43). Kesemua primer (kecuali OPD-09) menunjukkan fenotip yang dikongsi samada bagi baka bebiri rambut atau baka bebiri bulu.

Analisa varians molekular menunjukkan empat daripada tujuh primer polimorfik (OPD-11, OPD-12, BRL-03 dan BRL-06) dapat mengesan varians bererti antara jenis *fleece*, antara populasi dan antara individu-individu dalam satu populasi. Primer OPD-03 dan BRL-02 tidak dapat mengesan varians bererti antara baka rambut atau bulu, manakala primer BRL-05 tidak dapat mengesan varians antara populasi-populasi. Primer OPD-11 mengesan varians yang paling tinggi antara individu-individu (91.08%) manakala primer BRL-02 mengesan varians yang paling kurang antara individu-individu (8.46%). Data pada keseluruhan menunjukkan varians sebanyak 74% antara individu-individu dalam populasi, 20% antara populasi-populasi dan 4% antara jenis *fleece*.

Variasi genetik dan struktur empat populasi bebiri dikenalpasti melalui kaedah RAPD-PCR. Pengetahuan in amat penting dalam membentuk suatu program pembiakan atau pembiak-kacukan baka dalam usaha bagi meningkatkan industri bebiri.

CHAPTER I

INTRODUCTION

In recent years, the sheep population in Malaysia has been steadily increasing. This is largely due to the liberal importation of various hair and wool sheep breeds to complement the local indigenous sheep breed, the Malin. This has led to a large mix of gene pools with little or no genetic information about some of these breeds. Lack of genetic information would hamper efforts to plan strategic breeding programmes in order to maximise yield. Therefore, a proper and systematic genetic evaluation of these breeds is necessary.

Before implementing any breeding and/or crossbreeding programs, it is imperative that the genetic variability within and between breeds be determined. This facilitates efficient sampling and utilisation of resources. The breeder can use this genetic information to make informed decisions regarding the choice of genotypes to cross for the development of the breed or to facilitate the identification of diverse parents to cross in order to maximise heterosis or hybrid-vigour.

Lack of information about the genetic background can also lead to the loss of genetic variability or diversity. Loss of genetic diversity cannot be regenerated since variation within a species is the result of very long evolutionary process. Population that shows higher genetic variability is considered an important genetic resource as they have greater potential for improvement and serve as invaluable resource for different selection criteria, especially when planning breeding programs.

The estimation of genetic variation is often limited by the availability of polymorphic markers. Traditionally, variation has been studied using morphological characters but with limitations. The use of isozymes to study variation brought renewed interest in this field, however it was the use of DNA markers that led to a phenomenal increase of studies in population genetics.

DNA techniques based on restriction endonuclease digest and Southern hybridisation, i.e., restriction fragment length polymorphism (RFLP) and variable number of tandem repeat (VNTR) are usually technically demanding and expensive. While, the use of microsatellite technique is often hindered by the lack of availability of DNA sequence information of the microsatellites and its lengthy protocol.

In 1990, Williams *et al.* described a simple method for assessing genetic variability based on the amplification of random DNA segments with primers of arbitrary nucleotide sequence. The genetic polymorphisms observed have been termed as random amplified polymorphic DNA (RAPD)

markers. These RAPD markers have been successfully applied to assess genomic variability in livestock such as cattle, sheep and poultry.

This study was designed to investigate the genetic variability of four breeds/breed groups of sheep namely, the Santa Ines, Barbados Blackbelly, Malin and the Dorset-Malin cross using the RAPD technique.

Objectives

The objectives of this study were to use DNA fingerprints produced by RAPD markers:

i. To identify specific polymorphic markers in sheep.

ii. To estimate the level of genetic distance and identify the cluster relationships within each population of sheep.

iii. To estimate the level of genetic distance and identify the cluster relationships between the four populations.

iv. To estimate the amount of variance between the hair and wool sheep detected by each of the chosen primer.

CHAPTER II

LITERATURE REVIEW

Sheep Nomenclature

The sheep is an ungulate mammal belonging to the order Artiodactyles, suborder Pecora and family Bovidae. It is grouped into the subfamily Caprinae due to its heavy and coiled horns. This subfamily is claimed to have originated in upland and mountainous regions of western Asia and south eastern Europe and their eventual domestication led to isolated groups that developed into numerous species, sub-species, and localised breeds (Devendra and McLeroy, 1982).

A total of seven distinct wild forms, divided into some 40 different breeds have been recognised in sheep. The genus *Ovis* includes all sheep, while domesticated sheep belong to the species *Ovis aries* (Devendra and McLeroy, 1982; Gatenby, 1986).

Sheep Breeds

The domestic sheep vary in size, shape of the body, nature of coat cover, productive ability, reproductive capacity, adaptability to different environmental conditions, and size and shape of appendages such as horns, tails and ears. As such, numerous methods have been used for classification of sheep; tail type, coat cover, body type and primary function are the commonest categories used. Within this broad classification they are further subdivided into breeds (Devendra and McLeroy, 1982; Gatenby, 1986).

A breed may be defined as a group or population of animals so linked by ancestry that their primary identifying characteristics are generally passed from parent to offspring in an uniformed manner. The term cannot be strictly adhered to for tropical sheep since they exhibit some percentage of morphological variation within a breed. Nevertheless, since breed purity refers to ancestry or lineage rather than to genetic purity in the strict sense of homozygosity, this variation can be largely disregarded (Devendra and McLeroy, 1982).

The classification based on coat cover or type of fleece produced is the most distinct and useful when describing or comparing sheep over large geographical area. Domestic sheep can be divided into three main groups according to their coat-cover: wool sheep, hair sheep and fur bearing sheep (Devendra and McLeroy, 1982). Table 1 shows major breeds of sheep according to their coat cover and primary use.

Wool Sheep

These may be divided into two types. Firstly, those yielding apparel or clothing fibres; this includes mainly the long-thin-tailed varieties of European breeding. The other is those producing coarse wool suited to carpet or floor-covering manufacture. This includes mainly the fat tailed/fat-rumpled breeds of the temperate and sub-tropical zones.

Hair Sheep

These are primarily found in the tropics, namely, western Africa, South America and the West Indies. The coat-cover has almost no commercial value, however, the skin has greater value and use than that of the wool sheep.

Fur-bearing Sheep

These produce coarse wool suitable for carpet making. However, they are noted for the pelt of the new-born lambs, which is prized for producing coats and headwear.

Table 1: Major Types and Breeds of Sheep According to Primary Use.

Primary Use	Coat Cover	Breeds
Mutton	Medium-wool	Southdown, Hamshire, Corridale, Dorset
	Long-wool	Leicester, Lincoln, Romney, Marsh, Cotswold
	Carpet-wool	Awassi, Kurdi, Karaman, Sandjabi, Lohi, Rahmani
	Hair and skins	Kababish, Ouda, Hejazi, Blackhead, Nellore, Africander
Wool	Fine-wool	Spanish Merino, Australian Merino, Rambouillet
Fur Lamb pelts	Fur-bearing	Karakul, Grey Shirazi, Kuche
Milk	Wool	Finnish Landrace, East Fresian, Texel, improved Awassi

Note: Modified from Devendra and McLeroy, 1982

Current Status of Sheep Breeding in Malaysia

In Malaysia, sheep production, which is mostly carried out by smallholders, lags behind the poultry and swine sectors, which are well-developed commercial enterprises. Nevertheless, increased interest in sheep farming to meet the demand for mutton and to increase the level of self-sufficiency has led to a major shift towards commercial production (Rajion *et al.*, 1993).

In the late 1980's, the National Advisory Committee (NAC) of the Department of Veterinary Services (DVS) established a goal of reaching a population of one million sheep by the year 2000. This involved massive