UNIVERSITI PUTRA MALAYSIA

DNA VARIATIONS WITHIN YAM CULTIVARS (DIOSCOREA SPP) AS INDICATED BY RAPD ANALYSIS

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By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

April 2000

Ever Dearest...

My beloved husband, my other half
Mohd Fauzi bin Badron
Your guidance, encouragement and
Most importantly your love
Are all the propelling power and
The guiding light for me
To taste the sweetness of success

For my baby, Muhammad Zakwan
My deepest love will always
Be with you in facing
The ups and downs of life
May you be the devout child

For my parents, umi and abah
Brothers and sisters
The love and deeds showered on me
Will always be in my mind

"This is the success to be shared by all of us....
The sacrifice and love will always be cherished in my heart.
Only Allah could repay for all your deeds..."

Amin.....

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

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Chairman: Associate Professor Dr. Sayed Mohd Zain b. Sayed Hasan

Faculty: Science and Technology

A research on the DNA variations in eighteen yam cultivars from Malaysia and Japan was conducted using RAPD method in order to identify the cultivars and evaluate their genetic relationship. A preliminary identification of the cultivars was made using morphological characters of the plants. Ten primers, which were capable of producing PCR amplifications, were used in this research and were able to produce 515 amplification bands.

Primer OPG-13 was found to be the best primer producing the most number and unambiguous fragments. All the ten primers were used for identifying and elucidating the genetic relationship among the cultivars. The dendrogram produced from the NTsys programme based on UPGMA, by using RAPD data

showed that a close genetic relationship with a high percentage of similarity is between accession UPM DA 023 with accession UPM DA 024. Accession UPM DE 007 is a different species that is of *Disocorea esculenta* and thus genetically distant from the other accessions of *Dioscorea alata*. The tuber and leaf morphology of *D. esculenta* is also different from those of *Dioscorea alata* and indeed their genome is different too.

The results of this research show that the RAPD technique can be used to identify the yam cultivars and to determine the genetic relationship among the cultivars. The genotype of all cultivars from Japan can be identified using only one primer, which is primer OPG-13. On the other hand, the identification of cultivars from Malaysia requires four primers, which are OPB-07, OPB-15, OPG-02 and OPG-13.

In general, there is a close genetic relationship among yam cultivars found in Malaysia. However, these cultivars are different from Japan cultivars with the similarity index as low as 31.3%, indicating a distant relationship between them. RAPD is a very useful technique for the determination of genetic relationship and identification of plant cultivars. Such technique was successfully employed in genetic relationship and identification studies of *D. alata* and *D. esculenta* cultivars in Malaysia.

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

MENGESAN VARIASI DNA DI ANTARA KULTIVAR YAM (*DIOSCOREA* SPP) MENGGUNAKAN ANALISIS RAPD

Oleh

KHAIRULHASNI BINTI MAT ISA

April 2000

Pengerusi: Profesor Madya Dr. Sayed Mohd Zain bin Sayed Hasan

Fakulti: Sains dan Teknologi

Satu kajian ke atas variasi DNA dalam lapan belas kultivar yam daripada Malaysia dan Jepun telah dijalankan dengan menggunakan kaedah RAPD untuk pencaman kultivar dan menilai pertalian genetik antara mereka. Pencaman awal kultivar telah dibuat berdasarkan kepada sifat-sifat morfologi tumbuhan tersebut. Sebanyak sepuluh primer yang mampu memberikan hasil amplifikasi PCR telah digunakan dalam kajian ini dan menghasilkan 515 jalur amplifikasi.

Primer OPG-013 didapati sebagai primer yang terbaik menghasilkan fragmen terbanyak dan jelas. Semua sepuluh primer digunakan untuk pencaman dan menjelaskan pertalian genetik antara kultivar-kultivar. Dendrogram yang dihasilkan dari analisis Ntsys, berdasarkan UPGMA, dengan menggunakan

data RAPD menunjukkan bahawa pertalian genetik yang rapat dengan peratus persamaan tertinggi ialah antara aksesi UPM DA 023 dengan aksesi UPM DA 024. Aksesi UPM DE 007 merupakan spesies berbeza, iaitu *Dioscorea esculenta,* oleh itu ianya mempunyai pertalian genetik yang jauh dengan lain-lain aksesi *D. alata*. Morfologi tuber dan daun *D.esculenta* adalah berbeza dengan *Dioscorea alata* dan sudah tentu sifat genetiknya juga adalah berbeza.

Hasil kajian ini menunjukkan teknik RAPD boleh digunakan ke atas yam untuk pencaman kultivar yam dan menentukan hubungan genetik antara kultivar-kultivar tersebut. Kesemua kultivar dari Jepun dapat dikenalpasti genotipnya dengan menggunakan satu primer sahaja iaitu primer OPG-13, manakala pencaman kultivar dari Malaysia memerlukan empat primer iaitu OPB-07, OPB-15, OPG-02 dan OPG-13.

Secara keseluruhannya, terdapat pertalian genetik di antara kultivar-kultivar yam yang dijumpai di Malaysia, tetapi agak berlainan dengan kultivar-kultivar dari Jepun dengan indeks persamaan yang rendah (31.3%) menunjukkan pertaliannya yang longgar. Teknik RAPD merupakan teknik yang sangat berguna untuk tujuan penentuan hubungan genetik dan pencaman kultivar tumbuhan. Teknik tersebut telah berjaya digunakan dalam kajian pertalian genetik dan pencaman kultivar-kultivar *D. alata* dan *D. esculenta* di Malaysia.

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LIST OF ABBREVIATIONS

bp	:	base pair
dATP	:	deoxyadenine-5'-triphosphate
dCTP	:	deoxycytidine-5'-triphosphate
dGTP	:	deoxyguanocine-5'-triphosphate
DNA	:	deoxyribonucleic acid
dNTP	:	deoxynucleotide triphosphate
dTTP	:	deoxythymidine-5'-triphosphate
EDTA	:	Ethylenediamine tetra-acetic acid
Kb	:	kilobase
$MgCl_2$	:	Magnesium chloride
OD	:	Optical density
PCR	:	Polymerase Chain Reaction
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
Taq	:	*Thermus aquaticus*
TBE	:	Tris-borate-EDTA
TE	:	Tris-EDTA
FAO	:	Food and Agriculture Organization

CHAPTER I

INTRODUCTION

Yam is a group of monocot plants belonging to the genus *Dioscorea* in the family Dioscoreaceae (Ayensu, 1972). The genus *Dioscorea* contains more than six hundred species (Hahn *et al*., 1987), but there are only six species are cultivated for food source. These include *D. alata, D. rotundata*, *D. dumetorum*, *D. bulbifera, D. cayenensis* and *D. esculenta.* Nevertheless, some wild species of yams can still be used as an important component for human diet (Miège, 1952; Hamon *et al.*, 1988). Some wild species of yam have medicinal properties and chemicals content found in them can be extracted for multiple medicinal purposes (Purseglove, 1983).

Dioscorea alata, the greater yam is one of the species widely cultivated in the tropical region, particularly in Asia, Africa and Central America. In Malaysia, it is categorized under tuber crop and known by the vernacular names as 'Ubi Badak ' or 'Ubi Besar'.

Yam (*D. alata*) is the staple foodstuff for millions of peoples in many tropical and subtropical countries and are a secondary food for many millions more throughout the world (Onwueme, 1978). It is an important source of food energy for humans in the humid and subhumid tropics of West, Central and East Africa in particular, and in other tropical areas of South-East Asia, the Carribean and parts of India, Japan and China (Okoli, 1988).

In Malaysia, this crop is only cultivated as a mixed crop in small area by villagers for local market especially in the East Coast of the Peninsula (Hasan and Lasim, 1996). Its nutritional value is not well-known by many people in Malaysia because research activities on this crop in Malaysis is still lacking. Although yam is believed to be originated in this region, it is not well known and less widespread in this country. Thus, yam germplasm in this country needs to be collected, documented and preserved to avoid extinction and to utilize in the crop improvement. Knowledge of the basic biology of yam is still very limited and taxonomic analysis is very confusing (Terauchi, 1995).

Growing food deficits are of great concern to all who are interested in the welfare of millions of people living in poverty in the third world (Osagie, 1992). Attempts to resolve the problems of food production have normally placed emphasis on increasing the production and productivity of grain crops, but little attention has been given to the tuber crops such as yams. The reason for this may be due to the several difficulties related to the storage, planting, disease control and processing of this crop.

The possibilities of improving the agronomic characteristics and food qualities of yam are good, but germplasm must be collected, evaluated, maintained and used for creating source of populations for genetic improvement (Okoli, 1988). In 1918, an effort has been made to bring in genetic resources of yam from other countries into Malaysia (Spring and Milsum, 1919), description and documentation of agronomic characteristics has also been made.

Nevertheless, this effort has not been preceded in order to bring up yam as a commercial crop (Ghani, 1989).

Yam cultivars can be distinguished based on morphological characters and its usefulness but the distinctness may overlap due to lack of genetic improvement and adaptation to the local conditions. With the development of the new molecular genetic technique to distinguish taxa at the lowest level, therefore such technique has increasingly been used to describe and distinguish cultivars within a collection of plant germplasm (Rao and Riley, 1994).

Random amplified polymorphic DNA (RAPD) is one of the molecular genetic technique that is very powerful for genotype identification, population and pedigree analysis and other genetic studies of the crops (Welsh and McClelland, 1990). The use of this marker in studying plant variation is becoming commonplace because the technique requires less labour and time compared with other molecular techniques (William *et al*., 1993). This technique has also been applied on several yam species for several purposes of the study.

RAPD analysis was carried out to detect DNA polymorphism in the improved clones and land races of cultivated yams such as *D. rotundata* and eleven other wild species of *Dioscorea* from Africa (Mignouna *et al*., 1995). RAPD method has been mentioned to be applied on yam species to differentiate 235 *D. alata* L. cultivars collected from all over the world which was able to

distinguish 15 group of tested yams into 28 different cultivars (Martin and Rhodes, 1977). Differentiations of yam cultivars and wild relatives have also been made using molecular genetic technique of allozyme analysis (Hamon *et al*., 1990; Terauchi, 1992;) and DNA fingerprinting (Kahl, 1995). These studies were useful for selecting wild yam species for crop breeding programmes (Terauchi, 1995).

Although RAPD technique has been reported to be used on yam group of plants, but there is no specific and detailed protocol of RAPD technique published in any paper or journal. Thus, research on yam using RAPD technique for genetic studies is still limited and required further improvement of such technique to gather genetic information of the crops.

The main objective of this study was to determine the genetic relationship of the yam cultivars collected in Malaysia and received from Japan using PCR-RAPD technology. The banding patterns obtained will also be used for identification of cultivars. The result of this genetic study will be useful in promoting the utilization of yam germplasm obtainable in Malaysia.

CHAPTER II

LITERATURE REVIEW

History and Geographical Distribution of Yam (*Dioscorea* spp)

The genus *Dioscorea* is one of the largest genera representing by taxa (Figure 1) distributed in all parts of tropical region of the world. *Dioscorea* spp are found almost everywhere in the tropic where the rainfall sufficient for their growth. They occur in both Old and New Worlds either in the wild state, or in the cultivated land. The areas where *Dioscorea* spp are not found include swamps, the near-desert district where the rainfall is less than 80 cm per annum, and high mountains with heavy frost. A considerable number of members of the genus have edible tubers, which occur in most parts of the tropics (Coursey, 1967).

The following taxonomic diagnosis (Figure 1), which is based on that of Burkill (1951), conveys the essential features of the nature of plants belonging to this family. Rhizomes producing annual shoots, which are twining except in dwarf species, the direction of twinning being specific. Flowers either hermaphrodite or dioecious, generally small, greenish and inconspicious, but often strongly scented. Ovary trilocular, inferior, sometimes separated from the perianth by a constriction: two ovules in each cells (in *Avetra* and *Rajania*, where the fruit

Order	:	Dioscoreales
Tribe	:	Dioscoreae
Family	:	Dioscoreaceae
Genus	:	Dioscorea
Species	:	*Dioscorea alata*
		Dioscorea esculenta

Plants with hermaphrodite flowers (Genus)

1. *Stenomeris* :Ovules, numerous in each loculus
2. *Avetra* :Ovules,two in each loculus. Climber.Fruit a samara.Five of the six ovules abort
3. *Trichopus* :Plant dwarf,with fleshy fruit. Plant dioecious (ovules always two in each loculus)
4. *Dioscorea* :Fruit a capsule
5. *Rajania* :Fruit a samara. Five of the six ovules abort
6. *Tamus* :Fruit a berry

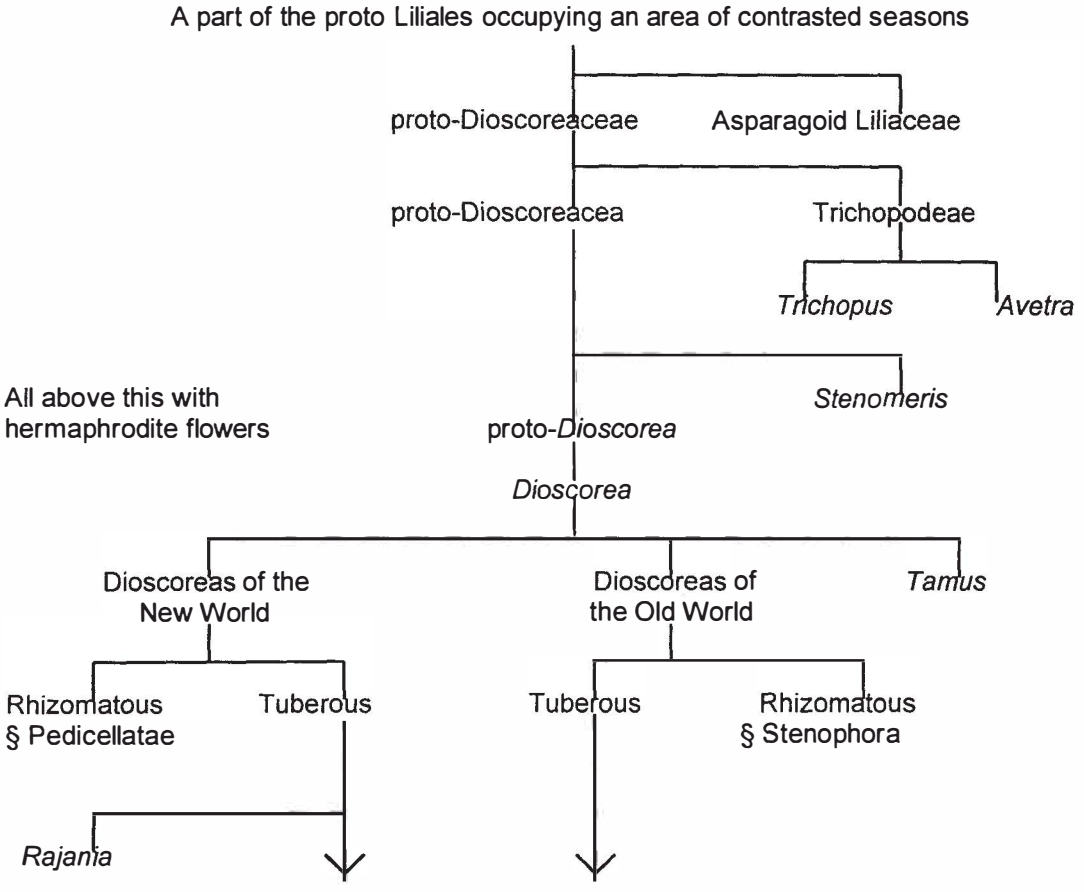

Figure 1: Position of the Genus of Dioscorea and its Satellite Genera in the Taxonomic Classification and Phyllogenetic Link of the Family Dioscoreaceae

Source: Burkill, 1960

is a samara, five of the six ovules (abort) except in *Stenomeris*, where they are numerous. Fruit either a capsule, a samara (*Avetra* and *Rajania*) or berry (*Tamus*). Seeds usually winged, but wingless in *Trichopus* and in a few species of *Dioscorea*, endosperm hard, embryo in marginal pocket. Illustration of morphology of certain part of *Dioscorea alata* is shown in Figure 2.

The family Dioscoreaceae was first recognized in 1819 by Brown under the name Dioscoreae, as including *Rajania* and *Tamus* along with *Dioscorea,* but it is only within the present century that it has been understood in its present form, the classification of such aberrant genera as *Trichopus* and *Stenomeris* presenting serious difficulties. The Dioscoreaceae are predominantly tropical plants and are distributed throughout those tropics except in the most arid areas. A few species inhabit the warmer parts of the temperate zones, but these are mostly of comparatively little economic importance (Coursey, 1967).

The relationships of the minor genera to *Dioscorea* are indicated in Figure 1, Burkill (1960), which also shows how the Dioscoreaceae are thought to have evolved out of the ancestral Liliales. The diagram shows that an American plants on the left. The predominance of satellite genera in the Old World strongly suggests that the Dioscoreaceae originated there: a view which is supported by the fact that the closest relations of the family are also Oriental. The greatest diversity of species, however, occurs in South America (Coursey, 1967).