



UNIVERSITI PUTRA MALAYSIA

**ISOLATION, CHARACTERIZATION AND APPLICATION OF DNA
MICROSATELLITE MARKERS IN MUNGBEAN (*VIGNA RADIATA* L.
WILCZEK) AND OTHER SELECTED LEGUMES**

VIJAY KUMAR

FSAS 2003 1

**ISOLATION, CHARACTERIZATION AND APPLICATION OF DNA
MICROSATELLITE MARKERS IN MUNGBEAN (*VIGNA RADIATA* L.
WILCZEK) AND OTHER SELECTED LEGUMES**

By

VIJAY KUMAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

January 2003



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**ISOLATION, CHARACTERIZATION AND APPLICATION OF DNA
MICROSATELLITE MARKERS IN MUNGBEAN (*VIGNA RADIATA* L.
WILCZEK) AND OTHER SELECTED LEGUMES**

By

VIJAY KUMAR

January 2003

Chairman: Prof. Dr. Tan Soon Guan

Faculty: Science and Environmental Studies

Mungbean (subgenus *Ceratotropis*) is an important food source in many parts of the world, particularly in Asia and Southeast Asia. It is an important source of plant protein and calcium, and is a good substitute for meat. Although it is an important crop, little is known about its genetic background. DNA markers, in particular microsatellites, are able to provide insights regarding the genetic structure and background of populations and thus would be of great benefit in mungbean improvement programs.

Three techniques were used to isolate microsatellite loci in mungbean, namely direct amplification of length polymorphism (DALP), 5' anchored PCR and random hybridizing microsatellites (RAHM). A total of 107 repeat sequences were identified of which 80% were microsatellite loci and 20% were cryptic simple regions. The majority of microsatellites were found using the 5' anchored PCR procedure which

proved to be the most efficient technique in the present study, while DALP did not produce any microsatellite.

Fourty-four microsatellite primer pairs were designed based on the mungbean DNA sequences obtained. Out of these, eight were unable to amplify the mungbean genome or gave irreproducible banding patterns and were subsequently discarded. The remaining 36 primer pairs gave excellent results upon polymerase chain reaction (PCR) amplification. In addition to mungbean-specific primers, six orthologous primer pairs from the common bean (*Phaseolus vulgaris*) were successfully used to amplify the mungbean genome. Thus, a total of 42 reproducible microsatellite markers were developed for use in mungbean.

Twenty-four primer pairs were used to evaluate the genetic variability in 11 populations representing three species of wild *Vigna* in Peninsular Malaysia. The three species of wild *Vigna* were *V. trinervia* (Beranang 1, Beranang 2, Banting, Triang, Bentong 1, Tangkak 1, Merapoh and Kg. Paya Mas), *V. reflexo-pilosa* (Bentong 2 and Tangkak 2) and *V. mungo* (Bukit Serdang). Primer pairs LR7315B and VJ3144B amplified two loci consistently, thus both these loci were scored independently, making the total number of loci scored for all the populations 26. The number of alleles per locus ranged from 1 to 12 with 4.6 as the average number. The total observed and expected heterozygosity across all 11 populations were 0.2858 and 0.4472, respectively. Characterization of the populations showed relatively high levels of genetic variation compared to previous studies using allozymes markers. Genetic distances were highest between populations of *V. trinervia* and *V. mungo*. Cluster analysis correctly differentiated the 11 populations according to their species.

Forty-two primer pairs were used to evaluate levels of genetic variability in 49 mungbean accessions. The number of alleles per locus ranged from 1 to 6 with an average of 2.2 alleles. The total observed and expected heterozygosity across all 49 mungbean accessions were 0.1676 and 0.2423, respectively. It was found that the genetic variability in the cultivated mungbean accessions was more than twice lower than in wild *Vigna* populations. This indicates the availability of genomic materials for introgression into cultivated mungbean. In the cluster analysis of mungbean accessions, no definite association between geographical origin and genetic distance was found.

The forty-two primer pairs developed for mungbean were also used to test for cross-amplifications in 15 other legume species. All the primers pairs were able to amplify the genomes of more than three legume species each. This indicates high levels of conservation among the DNA sequences of legumes. The successful cross-amplifications of mungbean primers in other legume species will save considerable amounts of time and valuable resources since the development of specific microsatellite markers for each legume species of interest is no longer a necessity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai keperluan ijazah Doktor Falsafah

**PEMENCILAN, PENCIRIAN DAN PENGGUNAAN PENANDA
MIKROSATELIT DNA DALAM KACANG HIJAU (*VIGNA RADIATA* L.
WILCEK) DAN LEGUM-LEGUM TERPILIH**

Oleh

VIJAY KUMAR

Januari 2003

Pengerusi: Prof. Dr. Tan Soon Guan

Fakulti: Sains dan Pengajian Alam Sekitar

Kacang hijau (subgenus *Ceratotropis*) merupakan sumber makanan yang penting di kebanyakan tempat di dunia, terutamanya di Asia dan Asia Tenggara. Ia merupakan sumber penting bagi protein tumbuhan dan kalsium, dan juga merupakan alternatif bagi daging. Walaupun ia merupakan suatu tanaman kontang yang penting, tidak banyak maklumat yang ada mengenai latarbelakang genetiknya. Penanda DNA, khususnya mikrosatelit, dapat memberi maklumat mengenai struktur genetik dan latarbelakang populasi dan justeru, membantu dalam program peningkatan kacang hijau.

Tiga teknik telah digunakan untuk memencil lokus mikrosatelit dari kacang hijau iaitu, *direct amplification of length polymorphism (DALP)*, *5' anchored PCR* dan *random hybridizing microsatellites (RAHM)*. Sejumlah 107 jujukan berulang telah dikenalpasti di mana 80% terdiri dari lokus mikrosatelit dan 20% adalah kawasan-

kawasan *cryptic simple*. Kebanyakan mikrosatelit dijumpai melalui teknik 5' *anchored PCR* di mana ia merupakan teknik yang paling berkesan dalam kajian ini, manakala DALP tidak menghasilkan sebarang mikrosatelit.

Empatpuluh empat pasangan primer mikrosatelit telah direka berdasarkan jujukan DNA kacang hijau yang diperolehi. Daripada jumlah ini, lapan primer tidak dapat mengamplifikasikan genom kacang hijau atau memberikan corak jalur yang tidak menyakinkan dan ia tidak digunakan. Baki 36 pasangan primer menghasilkan keputusan yang baik selepas amplifikasi tindakbalas rantai polimerase (PCR). Di samping primer spesifik kacang hijau, enam pasangan primer ortologus dari kacang biasa (*Phaseolus vulgaris*) telah berjaya digunakan untuk mengamplifikasi genom kacang hijau. Oleh itu, sejumlah 42 penanda mikrosatellit telah dihasilkan untuk kegunaan dalam kacang hijau.

Duapuluh empat pasangan primer telah digunakan untuk menilai variasi genetik dalam 11 populasi yang terdiri daripada tiga spesies liar *Vigna* di Semenanjung Malaysia. Ketiga-tiga spesies liar *Vigna* adalah *V. trinervia* (Beranang 1, Beranang 2, Banting, Triang, Bentong 1, Tangkak 1, Merapoh dan Kg. Paya Mas), *V. reflexo-pilosa* (Bentong 2 dan Tangkak 2) dan *V. mungo* (Bukit Serdang). Pasangan primer LR7315B dan VJ3144B mengamplifikasi dua lokus secara konsisten dan ia telah dicatat secara berasingan, menjadikan jumlah keseluruhan lokus yang dicatat dalam semua populasi sebanyak 26. Bilangan alel setiap lokus berjulat antara 1 hingga 12 dengan purata bilangan alel setiap lokus sebanyak 4.6. Jumlah heterozigositi cerapan dan jangkaan bagi kesemua 11 populasi adalah 0.2858 dan 0.4472, masing-masing. Pencirian populasi menunjukkan kadar variasi genetik yang tinggi berbanding kajian

sebelumnya yang menggunakan penanda alozim. Jarak genetik adalah paling tinggi di antara populasi *V. trinervia* dan *V. mungo*. Analisa kelompok telah dapat membezakan 11 populasi tersebut menurut jenis spesiesnya.

Empatpuluh dua pasangan primer telah digunakan untuk menilai kadar variasi genetik dalam 49 jenis kacang hijau. Bilangan alel setiap lokus berjulat antara 1 hingga 6 dengan purata bilangan alel setiap lokus sebanyak 2.2. Jumlah heterozigositi cerapan dan jangkakan bagi kesemua 49 jenis kacang hijau adalah 0.1676 dan 0.2423, masing-masing. Hasil keputusan menunjukkan variasi genetik dalam kacang hijau yang dikultur adalah dua kali ganda lebih rendah daripada populasi liar *Vigna*. Ini menunjukkan terdapatnya bahan-bahan genomik sedia ada untuk dipindahkan ke kacang hijau yang dikultur. Dalam analisis kelompok aksesori kacang hijau, didapati tiada hubungan antara asal-usul geografi dan jarak genetik.

Empatpuluh dua pasangan primer yang dihasilkan bagi kacang hijau telah digunakan untuk menguji amplifikasi-merentasi 15 jenis spesies legum. Kesemua pasangan primer dapat mengamplifikasi genom lebih daripada satu jenis spesies legum. Ini menunjukkan kadar pemuliharaan yang tinggi di antara jujukan DNA legum. Kejayaan amplifikasi-merentasi spesies legum dapat menjimatkan masa serta sumber penting memandangkan proses penghasilan penanda mikrosatelit khusus bagi spesies yang dikaji tidak lagi diperlukan.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Prof. Dr. Tan Soon Guan, Dr. Quah Soon Cheang and Prof. Datin Dr. Khatijah Yusoff. I am greatly indebted to each of them for their continuous support, cooperation and interest in my research and their comments and suggestions on many aspects of this work.

I would like to gratefully acknowledge the financial support from the Intensification of Research in Priority Areas (IRPA) project grant no. 09-02-04-004 and the Fundamental short-term research grant no. 05-11-02-0064S. I am deeply grateful to Dr. Quah Soon Cheang and Monash University Malaysia, Assoc. Prof. Dr. Siti Shapor Siraj and Prof. Datin Dr. Khatijah Yusoff for providing some extra financial support that allowed for the smooth running of the project.

I also want to gratefully acknowledge the Ministry of Science, Technology and the Environment Malaysia for providing me with a National Science Fellowship (NSF) Scholarship.

I would like to thank my three undergraduate students, Gillian Tsu, Hor Li Chian and Chong Siaw Chee who have helped me with the mungbean project and also made teaching an exciting experience for me.

My thanks also go to Dr. Sahar Usmani, Dr. Subha Bhassu, Chan Soon Choy, Hoh Boon Peng and Hisyam for their advice, friendship and the many interesting

discussions we had on microsattellites. Without them, life in the lab would indeed be boring and uneventful.

I would also like to thank my family for their moral support. I especially want to thank Dad for giving me strength, encouragement and for having faith in me.

I certify that an Examination Committee met on the 28th January 2003 to conduct the final examination of Vijay Kumar on his Doctor of Philosophy thesis entitled "Isolation, Characterization and Application of DNA Microsatellite Markers in Mungbean (*Vigna radiata* L. Wilczek) and Other Selected Legumes" 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:

Siti Shapor Siraj, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairperson)

Tan Soon Guan, Ph.D.

Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Quah Soon Cheang, Ph.D.

School of Science and Engineering
Monash University Malaysia
(Member)

Khatijah M. Yusoff, Ph.D.

Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Mahani Mansor Clyde, Ph.D.

Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Independent Examiner)



SHAMSHER MOHAMAD RAMADILI, Ph.D.

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 6 FEB 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Tan Soon Guan, Ph.D.

Professor

Faculty of Science and Environmental Studies

Universiti Putra Malaysia

(Chairman)

Quah Soon Cheang, Ph.D.

School of Science and Engineering

Monash University Malaysia

(Member)

Khatijah M. Yusoff, Ph.D.

Professor

Faculty of Science and Environmental Studies

Universiti Putra Malaysia

(Member)



AINI IDERIS, Ph.D.

Professor/Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 13 MAR 2003

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.



Vijay Kumar

Date: 6/03/2003

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	x
DECLARATION FORM	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF PLATES	xix
LIST OF ABBREVIATIONS	xxi
 CHAPTER	
 1 INTRODUCTION	 1
1.1 Objective	3
 2 LITERATURE REVIEW	 4
2.1 Taxonomic History of <i>Vigna</i>	4
2.1.1 Subgenus <i>Vigna</i>	5
2.1.2 Subgenus <i>Ceratotropis</i>	6
2.2 The Mungbean	7
2.2.1 Nomenclature of Mungbean <i>Vigna radiata</i> (L.) Wilczek	8
2.3 Mungbean in Malaysia	9
2.4 Mungbean Germplasm Relationship	10
2.5 Relationship of Mungbean and Other <i>Vigna</i> Species	12
2.6 Genetic Linkage Maps	13
2.7 Microsatellite Markers	15
2.7.1 Application of Microsatellites	16
2.8 Direct Amplification of Length Polymorphism (DALP)	19
2.9 Random Amplified Polymorphic DNA (RAPD)	20
2.10 Random Hybridizing Microsatellites (RAHM)	20
2.11 Five Prime Anchored PCR	21
 3 ISOLATION OF MICROSATELLITE LOCI	 22
3.1 Introduction	22
3.2 Methodology	24
3.2.1 Direct Amplification of Length Polymorphism	24
3.2.2 Five Prime Anchored PCR	32
3.2.3 Random Hybridizing Microsatellites (RAHM)	39
3.2.4 Submission of DNA Sequences to GenBank	43
3.2.5 Designing Primers Flanking Microsatellite Regions	43
3.2.6 Microsatellites from the Common Bean	44
3.3 Results	46
3.3.1 Isolation of Microsatellites	46
3.3.2 Microsatellite Primers	69
3.3.3 Orthologous Primers from Common Bean	69



4	POPULATION STUDY OF WILD <i>VIGNA</i>	73
4.1	Introduction	73
4.2	Methodology	75
4.2.1	Sampling Sites	75
4.2.2	Primers, PCR Conditions and Program	76
4.2.3	Gel Electrophoresis	79
4.2.4	Family Study	80
4.2.5	Data Analysis	80
4.3	Results	84
4.3.1	Wild Black Gram Population	84
4.3.2	Microsatellite loci amplification	84
4.3.3	Levels of Genetic Variation	87
4.3.4	Hardy-Weinberg Equilibrium Analysis	88
4.3.5	Genetic Distance and Cluster Analysis	112
4.3.6	Linkage Disequilibrium	115
4.3.7	Analysis of Population Subdivision	115
4.3.8	Family Study	116
5	MICROSATELLITE ANALYSIS OF CULTIVATED MUNGBEAN VARIETIES	118
5.1	Introduction	118
5.2	Methodology	121
5.2.1	Samples	121
5.2.2	Primers	121
5.2.3	PCR Amplification and Gel Electrophoresis	124
5.2.4	Data Analysis	124
5.3	Results	124
5.3.1	Description of Markers	124
5.3.2	Genetic Analysis of Mungbean Varieties	125
5.3.3	Linkage Disequilibrium	131
5.3.4	Test for Neutrality	131
5.3.5	Genetic Distance and Cluster Analysis	134
6	CROSS-SPECIES AMPLIFICATION OF MICROSATELLITE LOCI	138
6.1	Introduction	138
6.2	Methodology	140
6.2.1	Samples	140
6.2.2	Primers	141
6.2.3	PCR Amplification and Gel Electrophoresis	141
6.3	Results	145
6.3.1	PCR Amplification	145
6.3.2	Banding Pattern and Cross-amplification	145
6.3.3	Cluster Analysis	153
7	DISCUSSION	155
7.1	Introduction	155

7.2	Isolation of Microsatellite Markers	156
7.2.1	Microsatellite Isolation Using DALP	158
7.2.2	Microsatellite Isolation Using 5' Anchored PCR	159
7.2.3	Microsatellite Isolation Using RAHMs	161
7.2.3	Cryptically Simple Regions	162
7.2.4	Orthologous Primers	162
7.3	Population Study of Wild <i>Vigna</i>	163
7.3.1	Microsatellite Loci Amplification and Banding Patterns	163
7.3.2	Gel Electrophoresis	165
7.3.3	Heterozygosity Levels in Wild <i>Vigna</i>	166
7.3.4	Hardy-Weinberg Equilibrium	167
7.3.5	Linkage Disequilibrium (LD) in Wild <i>Vigna</i>	168
7.3.6	Microsatellite Inheritance Study of Microsatellite Markers	169
7.3.7	Genetic Distance and Cluster Analysis	170
7.4	Analysis of Cultivated Mungbean Varieties	171
7.4.1	Sample Size	172
7.4.2	Heterozygosity Levels in Mungbean	172
7.4.3	Genetic Distance in Mungbean	173
7.4.4	Test for Neutrality and Cluster Analysis of Mungbean	174
7.4.5	Classification of Variety V1160	176
7.5	Comparison Between Wild and Cultivated <i>Vigna</i>	176
7.6	Cross-amplification Study	179
7.6.1	Relationship of Mungbean and Other Legume Species	179
7.6.2	Cluster Analysis	181
7.6.3	Genetic Map	182
8	SUMMARY AND CONCLUSION	184
	REFERENCES	187
	APPENDIX	202
A.1	Microsatellite sequences of <i>Vigna radiata</i>	202
B.1	Microsatellite allele frequencies at 26 loci across 11 populations of wild <i>Vigna</i>	211
B.2	Linkage disequilibrium between pairs of microsatellite loci for Each population calculated using Burrow's composite measure of linkage disequilibrium	217
B.3	Microsatellite allele size and frequency at 42 loci of cultivated mungbean varieties	220
C.1	Silver staining protocol using the kit provided by Bio-Rad (USA)	223
D.1	Formulas used in the statistical analyses	224
	BIODATA OF THE AUTHOR	227

LIST OF TABLES

Table	Page
2.1 Linkage maps available for mungbean and other <i>Vigna</i> species.	14
3.1 Sequences of the DALP primers used in this study (Desmarius <i>et al.</i> , 1998).	27
3.2 Degenerate primers that were designed for 5' anchored PCR.	33
3.3 RAPD primers used for RAHMs.	40
3.4 Twelve microsatellite loci of <i>Phaseolus vulgaris</i> identified from the GenBank database (modified from Yu <i>et al.</i> , 1998).	45
3.5 The number of positive white colonies and the percentage of success.	54
3.6 List of microsatellites isolated from <i>Vigna radiata</i> .	62
3.7 Microsatellite loci in <i>V. radiata</i> , primer sequences and the expected PCR amplification product size.	70
3.8 Size of orthologous microsatellite alleles and their banding patterns.	72
4.1 Wild <i>Vigna</i> seeds from several sites in Peninsular Malaysia.	76
4.2 Microsatellite primers used for the population study.	83
4.3 Microsatellite variation in eleven populations of wild <i>Vigna</i> .	89
4.4 Number of observed and effective alleles and values of F_{is} statistics for all the loci.	100
4.5 Chi-square and likelihood ratio test for Hardy-Weinberg equilibrium for all populations.	101
4.6 Nei's (1978) unbiased genetic distance and identity based on microsatellite markers in eleven populations of wild <i>Vigna</i> .	113
5.1 Mungbean varieties and their place of origin.	123

Table	Page
5.2 Microsatellite loci heterozygosity and number of alleles in 49 accessions of <i>V. radiata</i> .	128
5.3 The overall Ewens-Watterson test for neutrality in 42 microsatellite loci.	132
6.1 Selected beans used in the cross-amplification study.	144
6.2 Cross-amplification of microsatellite markers developed for mungbean in 15 selected legume species.	147

LIST OF FIGURES

Figure	Page
3.1 Diagram showing the interpretation of the DALP profile of plate 3.1.	49
3.2 Microsatellite sequence obtained from automated DNA sequencer.	59
3.3 Cryptic simple sequence, clone LR121.	60
4.1 Map of Peninsular Malaysia indicating sampling sites of the wild <i>Vigna</i> populations.	77
4.2 A UPGMA dendrogram based on Nei's (1978) unbiased genetic distance showing relatedness among wild <i>Vigna</i> populations.	114
5.1 UPGMA dendrogram of 49 mungbean accessions using 37 microsatellite loci.	136
5.2 UPGMA dendrogram of 49 mungbean accessions based on 42 microsatellite loci.	137
6.1 UPGMA dendrogram showing the relatedness of the various bean legumes used in cross-amplification.	154

LIST OF PLATES

Plate		Page
3.1	Polyacrylamide gel electrophoresis of mungbean genomic DNA amplified with DALP235 and DALPR.	48
3.2	PCR re-amplification of DALP bands after excision from the polyacrylamide gel.	50
3.3	DNA sequence of band T38 obtained from the PCR amplification of DALP235 and DALPR.	51
3.4	Agarose gel (2%) electrophoresis of PCR product obtained from primer PCT4.	53
3.5	Agarose gel (2%) electrophoresis of PCR product obtained from amplification of variety V3476 using degenerate primers.	53
3.6	Colony hybridisation of LR7 clones with the microsatellite probe (GA) ₁₆ .	55
3.7	Plasmids of 5' anchored PCR clones.	57
3.8	Microsatellite sequence obtained from sequencing of PCT4 positive clone number 3(A) and 45(B).	58
3.9	RAPD primer OPA4 before and after hybridisation.	61
4.1	Morphology of wild <i>Vigna</i> seeds.	82
4.2	Microsatellite profile of wild <i>Vigna</i> samples from Kg. Paya Mas and Bentong 1 using primer pair LR733B.	89
4.3	Microsatellite profile of wild <i>Vigna</i> samples from Beranang 1, Beranang 2 and Tangkak 1 using primer pair LR1212B.	89
4.4	Microsatellite profile of wild <i>Vigna</i> samples from Triang, Merapoh and Banting using primer pair LR1212B.	90
4.5	Microsatellite profile of wild <i>Vigna</i> samples from Bentong 2 using primer pair LR7323B.	90

Plates	Page
4.6 Microsatellite profile of family study using primer pair LR738A.	121
5.1 Seed morphology of some of the mungbean varieties.	122
5.2 Microsatellite profile of mungbean varieties using primer pair LR733B.	126
5.3 Microsatellite profile of mungbean varieties using primer pair VJ3120B.	126
5.4 Microsatellite profile of mungbean varieties using primer pair LR121A.	127
5.5 Microsatellite profile of mungbean varieties using primer pair VJ31122A.	127
6. 1 Morphology of some of the <i>Vigna</i> seeds used in the cross-amplification study.	142
6.2 Microsatellite profile of some legume species using primer pair LR7319B.	146

ABBREVIATIONS

α	alpha
AFLP	amplified fragment length polymorphism
ATP	adenosine triphosphate
β	beta
bp	base pairs
Ci	Curie
dH ₂ O	distilled water
ddH ₂ O	double distilled water
deH ₂ O	deionized water
dNTP	deoxyribonucleotide
kb	kilobase
λ	lambda
LB	Luria-Bertani
mCi	millicurie
mM	millimolar
ng	nanogram
nmol	nanomole
PCR	polymerase chain reaction
pm	picomole
RAHM	random hybridising microsatellites
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
rpm	revolution per minute
μ g	microgram
μ l	microliter
μ M	micromolar
UPGMA	unweighted pair-group method with arithmetic mean
V	volts
w/v	weight/volume
xg	centrifugal force

CHAPTER 1

INTRODUCTION

Mungbean is an important food crop in the developing world, particularly in Asia where 98% of the world's mungbean is grown. It is an excellent and cheap source of plant protein and calcium, and is well-suited for cultivation throughout the tropics and subtropics. It is used for food, forage and green manure.

Although it is an important crop, little is known about the genetic background of mungbean. This lack of information has led to the loss of genetic variability or diversity in the plant. Such a loss cannot be regenerated since variation within a species is the result of very long evolutionary processes. Therefore, mungbean accessions that show high genetic variability are considered important genetic resources as they have greater potential for improvement and serve as invaluable resource for different selection criteria, especially when planning breeding or crossbreeding programs.

However, before implementing any breeding and/or crossbreeding programs, it is important that the genetic variability within and between accessions or varieties is determined. This would facilitate efficient sampling and utilisation of resources. The plant breeder can then use this genetic information to make informed decisions regarding the choice of genotypes to cross for the development of the cultivars or to facilitate the identification of diverse parents to cross in order to maximise heterosis or hybrid-vigour.

The estimation of genetic variation is often limited by the availability of polymorphic genetic markers. Traditionally, variation has been studied using morphological characters but with limitations. The use of isozymes to study variation brought renewed interest in the field and is still used by many researchers, as it is an efficient and cost-effective marker, however it was the use of DNA markers that led to a phenomenal increase of studies in population genetics.

Several types of DNA markers have been developed in recent years to assist in genetic analysis. These include Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphisms (AFLP), Random Amplified Polymorphic DNA (RAPD), Direct Amplification of Length Polymorphisms (DALP), Random Amplified Microsatellites (RAM), and Variable Number of Tandem Repeats (VNTR) such as minisatellites and microsatellites. In spite of the many types of markers available, the most efficient and effective marker system is microsatellites which is also known as simple sequence repeats (SSR). Unfortunately, microsatellite markers have not been developed in mungbean because of the difficulty of their isolation. As a result, other types of markers such as isozymes, RFLP, RAPD, RAM and proteinase inhibitors have been used for phylogenetic and taxonomic purposes in mungbean with varying degrees of success.

1.1 Objectives

The mungbean is a convenient plant for genetic studies due to several reasons. It has a short life cycle, is naturally self-pollinated and easily cross-pollinated, a diverse germplasm collection is available and it may possibly serve as a model system for other commercially important legumes.

The primary objectives of this study are to:

1. identify and isolate microsatellite markers in mungbean,
2. characterize the microsatellite markers,
3. apply these microsatellite markers to study the genetic structure of wild and cultivated *Vigna* species.

The specific objectives of this study are to:

1. develop efficient methodologies for the isolation of microsatellite markers in mungbean,
2. estimate levels of heterozygosity and genetic distance using microsatellite markers in wild *Vigna* species collected from Peninsular Malaysia and cultivated accessions of mungbean.
3. estimate phylogenetic relationships among cultivars of mungbean,
4. test for cross-amplification of microsatellite primers in closely related species,
5. evaluate the inheritance of microsatellites from parents to offsprings.