



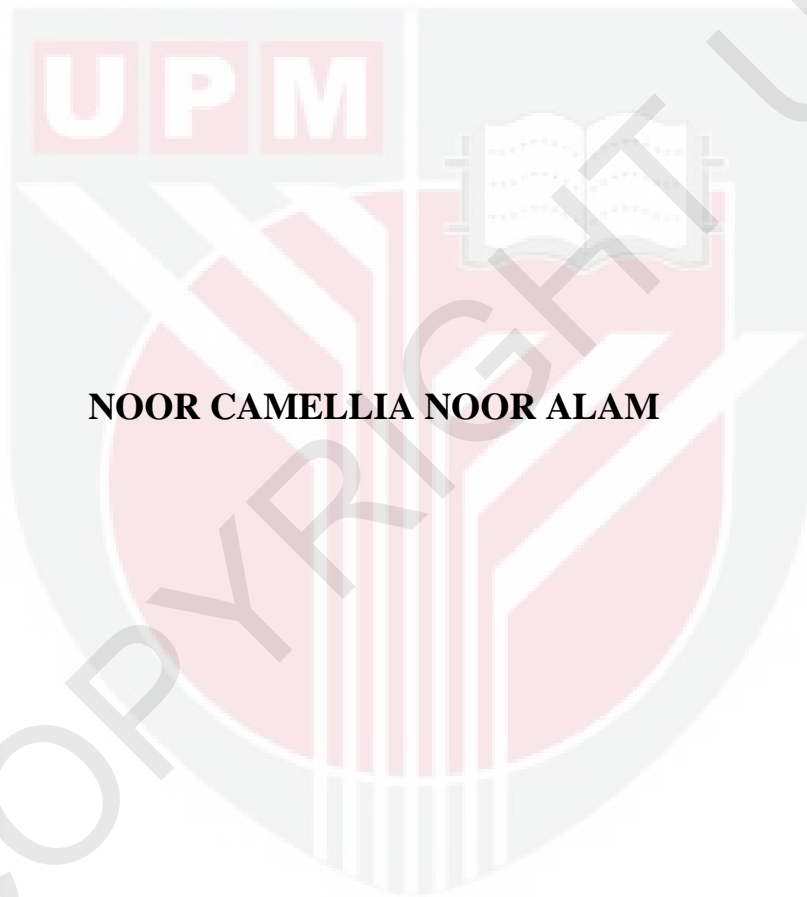
UNIVERSITI PUTRA MALAYSIA

***REPRODUCTIVE SYSTEM AND MOLECULAR VARIATIONS OF
JATROPHA CURCAS L. ACCESSIONS***

NOOR CAMELLIA NOOR ALAM

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**REPRODUCTIVE SYSTEM AND MOLECULAR VARIATIONS OF
JATROPHA CURCAS L. ACCESSIONS**



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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2012

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The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. At the top left, the letters 'UPM' are written in white on a red rectangular background. The central part of the shield features a stylized white bird or wing design. To the right of the bird is an open book with text on its pages. The shield is divided into several sections with different colors and patterns, including red, white, and grey.

**By
NOOR CAMELLIA NOOR ALAM**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
In Fulfilment of the Requirements for the Master of Science
May 2012**

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

**REPRODUCTIVE SYSTEM AND MOLECULAR VARIATIONS OF *JATROPHA*
CURCAS L. ACCESSIONS**

By

NOOR CAMELLIA NOOR ALAM

May 2012

Chairman: Associate Professor Thohirah Lee Abdullah, PhD

Faculty: Agriculture

Three studies were conducted to describe microscopically the flowering and fruit set, pollination ecology and genetic relationships and variations of *Jatropha curcas* L. accessions in Malaysia. For the first study, investigations were carried out by observing the floral morphology, flowering sequence of pistillates, floral anthesis time, flower daily anthesis, flowering and fruiting behaviour pattern, flower sex and fruit set ratio of *Jatropha curcas*. The organogenesis of the floral reproductive organs was examined using Scanning Electron Microscope (SEM). *Jatropha* is a monoecious plant and produces individual flowers in a dichasial cyme. Each *Jatropha* inflorescence has at least six compound dichasium. *Jatropha* flowers are pale green in colour with only a difference in sepal length between the male and female flowers. The flowering sequence of the female flowers in *Jatropha* begins at the upper most terminal of the inflorescence and simultaneously on the upper most terminal of the lowest cyme tier and this is followed by other cyme tier in the inflorescence for the second day of flowering. Male flower anthesis started the earliest at hour 0000 and again at hour 0610-0646 while the female flower anthesis commenced at 0635-0825. Male flowers anthesis

was for a period of 8-11 days, while female flowers opened for only 3-4 days. Two observations on flower sex and fruit set ratio were done at different flowering season in December 2008 and April 2009. The reading of the male to female flower ratio was 22: 1 in December 2008 and 27: 1 in April 2009. The flower to fruit ratios were 6: 5 (January 2009) and 2: 1 (May 2009). Numerically, 0-10 female flowers and 25-215 male flowers are produced in the same inflorescence. In this study, the terminal stem of *Jatropha* bears fruits profusely in January, May and August 2009. In March, June and October, flowering took place after the vegetative stage. Development of the floral meristem consists of three stages that include a vegetative stage, transition from vegetative to floral stage and the development of flower parts. The meristem was in the transition stage at day 6. All sepals and a petal were developed at day 18 but there was no presence of reproductive organs developing at this stage. Flower and fruit development takes approximately 100 days to complete the cycle from the initiated floral bud stage until fruit maturity. Continuous flowering on the same inflorescence and the incidents where flowering terminated in the middle of the flowering period were factors believed to cause the wide range of fruit ripening times recorded. The problem of small number of fruits produced in *Jatropha curcas* is mainly caused by the small number of pistillate flowers present in each inflorescence that range from 0 to 10 flowers in the each inflorescence. *Jatropha* could have two to four cyclical fruiting peaks in Malaysia. For the second study, investigation was carried out by observing the flower visitors behaviour based on the duration of each visit by an insect species, mean abundance, the peak hour of visitation, and the rate of visitation during 5 min periods (number of visits/min) throughout the day (24 hours) for ten plants. Eight insect species were found to visit *Jatropha curcas* flowers in this study which is *Monomorium pharaonis* L., *Monomorium minimum*, *Lasius niger*, *Apis cerana*, *Lucilia* sp., *Anomala pallid*, *Blattela germanica* and *Hexacentrus unicolor*. Ant species; *Monomorium pharaonis*, *Monomorium minimum* and *Lasius niger* can

be found abundantly at *Jatropha* flowers which contribute 92% of overall visitation. More than one individual species were observed visiting the flowers at a time except *Hexacentrus unicolor*. The numbers of insect visited male flowers (786 visits) were almost three times more than on female flowers (295 visits). The rate of visitation was almost two visits per minute per inflorescence. *Apis cerana* is the fastest among all species to visit *Jatropha* flowers which spent one to five seconds and observed robbing the *Jatropha* pollens. It visited male flowers at hour 0830 to 1400 and visited female flowers at hour 1300 to 1400. All species of ants and flies were only visiting for nectars. *Monomorium pharaonis* L. can be observed abundantly at most of the time from early morning till midnight to rob nectar. The study revealed that *Monomorium pharaonis*, *Monomorium minimum*, *Lasius niger* and *Apis cerana* can be considered as effective pollinators for *Jatropha curcas* with regard to the duration of visits and abundancy. The peak hour of visitation occurred during the day which around 0830 till 1830 right after the flower anthesis took place. This situation promotes pollination to the maximum point as insect pollinators can be found abundantly after the flower anthesis. For the third study, the experiment was undertaken to assess the extent of genetic diversity in a representative set of 16 accessions of *Jatropha curcas*. Inter-simple sequence repeat (ISSR) analysis was used to establish the genetic relationship among the accessions. Only six ISSR primers UBC812, UBC834, UBC847, UBC880, I1 and I2 generated a polymorphic banding profile out of 8 primers used. The number of amplicons per primers varied from 6 (UBC812) to 14(UBC834) and the amplicon size from 151 bp to 2779 bp. Out of a total of 55 bands, 25 (45.45%) were polymorphic with an average of 4.16 polymorphic bands per primer. Jaccard's coefficient of similarity varied from 0.72 to 1, indicating low level of genetic variation among the studied genotypes. The similarity was found to be the lowest (0.72) between accessions from Kelantan and Selangor while the highest (1.0) was between accessions from Sabah and Brunei. UPGMA cluster analysis indicated six main clusters at a threshold of 90% with the

highest number of accessions grouped under cluster II. Although, the grouping was not related to location sources, there was a close genetic relationship among the *Jatropha* accessions represented. The low degree of variation represented maybe mainly attributed to the fact that *J.curcas* is an introduced plant species in Malaysia and comes from the same source when they were introduced to Malaysia and most likely the source was to be Indian as two of the accessions (I & M) were from India. The variation also can low due to the low number of markers used in the study.



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

**SISTEM REPRODUKTIF DAN VARIASI MOLEKULAR BAGI *JATROPHA CURCAS* L.
AKSESI**

Oleh

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Mei 2012

Pengerusi: Profesor Madya Thohirah Lee Abdullah, PhD

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Tiga kajian telah dijalankan untuk menerangkan pembungaan dan pembentukan buah, ekologi pendebungaan dan hubungan genetik dan variasi aksesori *Jatropha curcas* di Malaysia. Dalam kajian yang pertama, pemerhatian telah dijalankan dengan memerhatikan morfologi bunga, urutan pembukaan bunga betina dalam satu jambak, masa pembukaan bunga, kelakuan pembungaan dan berbuah dan nisbah bunga mengikut jantina dan nisbah bunga kepada buah. Organ pembiakan bunga di perhati menggunakan mikroskop imbasan elektron (SEM). *Jatropha* adalah tumbuhan monoecious dan menghasilkan individu bunga dalam bentuk dikasial sima. Setiap jambak bunga *Jatropha* mempunyai sekurang-kurangnya enam kompaun dikasium. Bunga *Jatropha* berwarna hijau pudar dengan hanya mempunyai perbezaan pada panjang sepal diantara bunga jantan dan betina. Turutan pembungaan bunga betina *Jatropha* bermula di terminal paling atas bagi jambak bunga dan serentak pada terminal paling atas bagi tingkat cyme yang terendah dan ini diikuti oleh tingkat cyme yg lain dalam jambak bunga untuk pembungaan pada hari kedua. Pembukaan bunga jantan adalah bermula seawal jam 0000 dan pada jam 0610 hingga

0646 manakala pembukaan bunga betina bermula pada jam 0635 hingga 0825. Tempoh antesis bunga jantan bagi sejambak bunga mengambil masa 8 hingga 11 hari untuk tamat, manakala bunga betina pula mengambil masa tiga hingga empat hari sahaja. Dua pemerhatian ke atas nisbah jantina bunga dan pembentukan buah dilakukan musim pembungaan yang berbeza iaitu pada Disember 2008 dan Januari 2009. Nisbah bunga jantan kepada betina adalah 22: 1 pada Disember 2008 dan 27:1 pada bulan April 2009. Nisbah untuk bunga kepada buah adalah 6:5 pada Januari 2009 dan 2:1 pada Mei 2009. Sejumlah 0 hingga 10 bunga betina dan 25-215 bunga jantan di hasilkan dalam satu jambak bunga *Jatropha curcas*. Dalam kajian ini, hujung batang *Jatropha* bergantung dengan buah yang banyak pada Januari, Mei dan Ogos 2009. Dalam Mac, Jun dan Oktober, pembungaan berlaku selepas peringkat vegetative. Pembentukan meristem bunga terdiri daripada tiga peringkat iaitu vegetatif, peralihan dari vegetatif ke pembungaan dan pembentukan bahagian bunga. Pada hari ke 6, meristem berada pada peringkat peralihan ke pembungaan. Kesemua sepal dan petal telah terbentuk pada hari ke 18 tetapi kehadiran organ pembiakan belum lagi terbentuk pada peringkat ini. Pembentukan bunga dan buah mengambil masa kira-kira 100 hari untuk melengkapkan kitaran dari permulaan peringkat putik bunga sehingga kematangan buah. Pembungaan berterusan pada sejambak bunga dan kejadian di mana pembungaan terhenti semasa tempoh pembungaan merupakan faktor yang dipercayai menyebabkan terdapat jarak masa yang besar antara masa kematangan buah yang direkodkan. Masalah penghasilan buah yang sedikit oleh *Jatropha curcas* adalah disebabkan oleh bilangan bunga betina yang sedikit dalam setiap jambak yang hanya terdiri dari 0 hingga 10 bunga betina sahaja. *Jatropha* mempunyai dua ke empat puncak kitaran berbuah di Malaysia setahun. Dalam kajian kedua, penyiasatan telah dijalankan dengan memerhatikan kelakuan pengunjung bunga berdasarkan tempoh setiap kunjungan bagi setiap spesies serangga, anggaran lambakan serangga,

waktu puncak lawatan dan kadar lawatan dalam tempoh masa 5 minit (bilangan kunjungan/ minit) sepanjang hari (24 jam) untuk sepuluh pokok. Lapan spesies serangga telah ditemui melawat bunga *Jatropha curcas* dalam kajian ini iaitu *Monomorium pharaonis* L., *Monomorium minimum*, *Lasius niger*, *Apis cerana*, *Lucilia* sp., *Anomala pallida*, *Blattella germanica* and *Hexacentrus unicolor*. Spesies semut seperti *Monomorium pharaonis*, *Monomorium minimum* dan *Lasius niger* boleh di temui dengan banyak pada bunga *Jatropha* yang menyumbang kepada 92% daripada keseluruhan lawatan. Lebih dari satu spesies individu diperhatikan melawat bunga pada satu-satu masa kecuali *Hexacentrus unicolor*. Bilangan serangga yang melawat bunga jantan (786 visits) adalah hampir tiga kali lebih daripada bunga betina (295 visits). Kadar lawatan adalah hampir dua kunjungan setiap minit bagi setiap jambak bunga. *Apis cerana* adalah species paling laju antara semua species yang melawat bunga *Jatropha* dengan meluangkan masa satu hingga lima saat dan telah diperhatikan mencuri pollen *Jatropha*. Ia melawat bunga jantan pada jam 0830 hingga 1400 dan melawat bunga betina pada jam 1300 hingga 1400. Semua species semut dan lalat hanya melawat untuk nectar. *Monomorium pharaonis* L. boleh diperhatikan dengan banyak di kebanyakan masa dari awal pagi hingga tengah malam untuk mencuri nektar. Kajian ini mendedahkan bahawa *Monomorium pharaonis*, *Monomorium minimum*, *Lasius niger* dan *Apis cerana* boleh dianggap sebagai pendebunga berkesan untuk *Jatropha curcas* dengan mengambil kira tempoh lawatan dan lambakan serangga. Waktu puncak bagi lawatan serangga berlaku pada siang hari sekitar jam 0830 hingga 1830 sejurus selepas pembukaan bunga berlaku. Situasi ini menggalakkan pendebungaan berlaku secara maksimum kerana serangga pendebunga boleh didapati dengan banyak selepas pembukaan bunga. Dalam kajian ketiga, percubaan telah dijalankan untuk menilai sejauh mana kepelbagaian genetik dalam set wakil 16 akses *Jatropha curcas*. Analisis Inter Simple Sequence Repeat (ISSR) telah

digunakan untuk mewujudkan hubungan genetik antara aksesori. Hanya enam ISSR primer, UBC812, UBC834, UBC847, UBC880, I1 dan I2 menghasilkan profil jalur polimorfik daripada lapan primer yang digunakan. Bilangan amplicon bagi setiap primer adalah berbeza dari 6 (UBC812) hingga 14(UBC834) dan saiz amplicon dari 151bp hingga 2779bp. Daripada jumlah 55 jalur yang diperolehi, 25 (45.45%) adalah jalur polimorfik dengan purata 4.16 jalur polimorfik bagi setiap primer. Persamaan pekali Jaccard berbeza dari 0.72 ke 1, menunjukkan tahap variasi yang rendah antara genotip yang dikaji. Persamaan ditemui paling rendah (0.72) antara aksesori dari Kelantan dan Selangor manakala yang paling tinggi (1.0) adalah antara aksesori dari Sabah dan Brunei. Analisis UPGMA menunjukkan terdapat enam kelompok utama pada ambang 90% dengan bilangan tertinggi aksesori berkumpul di bawah kelompok II. Walaupun kumpulan itu tidak berkaitan dengan lokasi sumber, terdapat hubungan genetik yang rapat di antara aksesori *Jatropha* yang diwakili. Tahap variasi yang rendah didapati mungkin disebabkan oleh hakikat bahawa *J.curcas* adalah spesies tumbuhan yang diperkenalkan di Malaysia dan juga datang dari sumber yang sama apabila diperkenalkan pada Malaysia dan kemungkinan sumber adalah dari India kerana dua dari aksesori (I & M) adalah dari India. Variasi yang rendah juga boleh disebabkan oleh penggunaan penanda molekular yang kurang dalam kajian ini.

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I certify that a Thesis Examination Committee has met on **14th May 2012** to conduct the final examination of **Noor Camellia Noor Alam** on her thesis entitled “**Reproductive system and molecular variations of *Jatropha curcas* L. accessions**” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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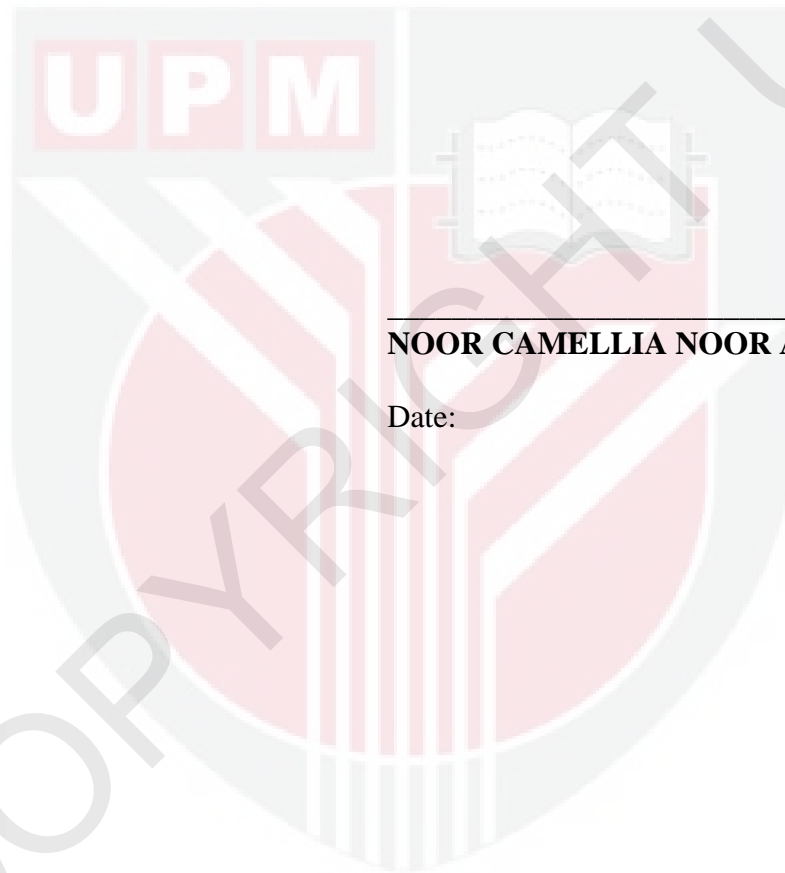
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



NOOR CAMELLIA NOOR ALAM

Date:

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vii
ACKNOWLEDGEMENTS	xi
APPROVAL	xii
DECLARATION	xiv
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	6
2.1 Physic Nut (<i>J. curcas</i>)	6
2.1.1 Origin and Distribution	6
2.1.2 Ecology	7
2.1.3 Morphological Description	8
2.1.4 Propagation	10
2.1.5 Potential Uses	10
2.1.5.1 Oil and Biodiesel	12
2.1.5.2 Medicinal	12
2.1.5.3 Poisonous Plant	13
2.1.6 Flowering and Fruiting	14
2.1.7 Pollination Ecology	16
2.2 Molecular Markers in Plant Studies	17
2.2.1 Molecular Markers for Genetic Analysis	17
2.2.2 Application of Microsatellite marker in genetic variation study	19
2.2.3 Genetic diversity of <i>J. curcas</i>	20
2.2.4 Molecular Genetic studies in <i>J. curcas</i>	21
2.2.5 Genetic Improvement of <i>J. curcas</i>	22
3 GENERAL MATERIALS AND METHODS	24
3.1 Study 1: Flowering and Fruit Set of <i>Jatropha curcas</i> L. Under Malaysian Climatic Condition	25
3.1.1 Experimental Site	25
3.1.2 Planting Material	26
3.1.3 Floral Biology and Flowering Pattern	26
3.1.3.1 Inflorescence and Floral Morphology	26
3.1.3.2 Flowering Sequence of Pistillates	26
3.1.3.3 Floral Anthesis Time	27
3.1.3.4 Flower Daily Anthesis	27
3.1.3.5 Flower Sex and Fruit Set Ratio	28
3.1.4 Floral and Fruit Development	28
3.1.4.1 Flowering and Fruiting Plant Behaviour	28
3.1.4.2 Detection of Floral Initiation	28

	3.1.4.3 Sample Preparation For Scanning Electron Microscope	29
3.2	Study 2: Pollination Ecology of <i>J. curcas</i> in Malaysia	30
	3.2.1 Experimental Site	30
	3.2.2 Planting Material	31
	3.2.3 Floral Visitors Observation	31
	3.2.3.1 Duration of Each Visit by Insect Species	31
	3.2.3.2 Mean Abundance	31
	3.2.3.3 The Peak Hour of Visitation	32
	3.2.3.4 The Rate of Visitation	32
3.3	Study 3: Genetic Relationships and Variation of <i>J. curcas</i> Accessions in Malaysia	32
	3.3.1 Experimental Site	32
	3.3.2 Planting Material	33
	3.3.3 Leaves Sampling Collection	34
	3.3.4 DNA Extraction	34
	3.3.5 Polymerase Chain Reaction and Primers	34
	3.3.6 Data Analysis	35
4	FLOWERING AND FRUIT SET OF <i>JATROPHA CURCAS</i> L. UNDER MALAYSIAN CLIMATIC CONDITION	36
	4.1 Introduction	36
	4.2 Materials and Methods	37
	4.3 Results	38
	4.3.1 Floral Biology and Flowering Behaviour	38
	4.3.2 Floral and Fruit Development	50
	4.4 Discussion	53
	4.5 Conclusion	55
5	POLLINATION ECOLOGY OF <i>J. CURCAS</i> IN MALAYSIA	57
	5.1 Introduction	57
	5.2 Materials and Methods	57
	5.3 Results	59
	5.4 Discussion	65
	5.5 Conclusion	67
6	GENETIC RELATIONSHIPS AND VARIATION OF <i>J. CURCAS</i> ACCESSIONS IN MALAYSIA	69
	6.1 Introduction	69
	6.2 Materials and Methods	73
	6.2.1 Plant Material	73
	6.2.2 DNA Extraction and PCR Amplification	75
	6.2.3 Data Analysis	77
	6.3 Results	78
	6.3.1 DNA Concentration and Quality	78
	6.3.2 Polymorphisms Detected by ISSR	78
	6.3.3 Genetic Relationships among Sixteen Accessions of <i>Jatropha curcas</i> L.	83

6.4	Discussion	85
6.5	Conclusion	87
7	GENERAL CONCLUSION AND RECOMMENDATIONS	88
	REFERENCES	92
	APPENDICES	98
	BIODATA OF STUDENT	121
	LIST OF PUBLICATIONS	122
	JOURNALS	122
	INTERNATIONAL AND NATIONAL CONFERENCE	123
	PROCEEDINGS	

