UNIVERSITI PUTRA MALAYSIA

GENETIC AND MOLECULAR ANALYSES FOR SEED AND OIL YIELDS IMPROVEMENT IN JATROPHA CURCAS L. POPULATIONS

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FP 2012 83

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BY

ALIREZA BIABANIKHANKAHDANI

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

April 2012

DEDICATIONS

To my beloved parents for
sacrifices, understanding and tremendous encouragement and support
throughout my study

To my sisters
for their kindness and taking care

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

GENETIC AND MOLECULAR ANALYSES FOR SEED AND OIL YIELDS IMPROVEMENT IN *JATROPHA CURCAS* L. POPULATIONS

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Chairman : Associate Professor Mohd Rafii Yusop, PhD

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The current Jatropha improvement program was designed with the main objective to produce superior planting materials with high seed and oil yield production for commercial planting. The specific objectives were to study inter and intra-populations variation using morphological and Inter Simple Sequence Repeat (ISSR) molecular markers, to estimate genetic components, heritability values, general and specific combining abilities of several important characters for identifying the best parental lines and finally to quantify the level of heterosis for F_1 hybrids in diallel crosses. Jatropha populations from Malaysia, Indonesia, India and Philippines were used as a base population in this selection program.

Sixty four plants in each of six Jatropha populations, namely Malaysia1 (My1), Malaysia2 (My2), Indonesia1 (In1), Indonesia2 (In2), India1 (Id1) and Philippines1

(Ph1) were planted for field evaluation in Serdang University Agriculture Park, Universiti Putra Malaysia. Jatropha populations were arranged in a randomized complete block design (RCBD) with four replications at spacing of 4 m × 2 m. Analysis of variance in seed characters revealed that there was significant variation among Jatropha populations for seed length, seed width, seed weight and 100-seed weight at $P \leq 0.05$. Population of My2 produced the highest oil content for both years (31.73 and 33.41%) of yield production. The lowest oil content was observed in population In1 for first and second years of harvesting (29.09 and 30.61%, respectively).

Significant variation ($P \leq 0.05$) was observed in plant height, number of secondary branches, days to flowering, number of inflorescences and seed yield. The highest seed yield was recorded in population In1 for first (46.5) and second year (222.8 g) in both years, whereas, the lowest was population In2 for first year and My1 for second year harvesting. Seed yield correlated significantly with days of flowering, number of inflorescences and plant height. Seed yield also correlated significantly with oil content. Days of flowering was found to have a high significantly positive relationship with plant height, number of secondary branches, number of tertiary branches, number of inflorescence, seed length and seed weight. The highest heritability was estimated for 100-seed weight (77.3%). The heritability values for plant height, number of secondary branch, days to flowering, seed weight, seed yield and oil content were 40.8, 47.8, 31.7, 77.2, 52.0 and 49.7% respectively.

ISSR markers were used to detect inter and intra-population variation of the based populations. The percentage of polymorphic bands for each population ranged from

46.15 to 60.84%, with an average of 55.10%. Among populations, the average number of alleles per locus (n_a) ranged from 1.46 (My1) to 1.61 (Ph1) with the mean number of 1.55, which the effective number of alleles per locus ($n_{e)}$ ranged from 1.12 (My1) to 1.17 (My2) with the mean number of 1.15. Among the populations, population My1 had the lowest genetic diversity levels, while population Ph1 showed the highest genetic diversity. The partitioning of genetic diversity into within population and between populations based on Shannon's diversity index also revealed more variation within populations (0.81) than variations between populations (0.19).

From the field evaluation of the Jatropha populations of 364 plants, ten superior individual plants (three from Malaysia, three Indonesia, two India and two Philippine population) were selected and were intercrossed in a half-diallel mating design (10×10) including selfed of each selected parental plants. The 45 F_1 hybrids and 10 selfed progenies were evaluated in field using a randomized complete block design (RCBD) with three replications in Puchong University Agriculture Park, Universiti Putra Malaysia. Analyses of variance showed significant ($P \leq 0.01$) variations among parents and hybrids for all characters. Analysis of variance for gene effects showed that both general (GCA) and specific combining ability (SCA) were influential for all traits. Analysis of specific combining ability in this study showed that a number of hybrids showed more precise for each character in different individual hybrids, but none showed the best SCA effect for all traits in one particular hybrid.

The percentages of heterosis for traits in some hybrids were considerably high indicating that a high degree of genetic diversity among parents. High broad sense heritability was recorded for all traits. However, the ratios of GCA/SCA indicated that non-additive effects were more important than additive gene effects. The analyses of GCA did not show that any single parent was a high general combiner for all traits simultaneously.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Doktor Falsafah

ANALISA GENETIK DAN MOLEKULAR UNTUK KEMAJUAN HASIL BIJI DAN MINYAK DI DALAM POPULASI *JATROPHA CURCAS* L.

Oleh

ALIREZA BIABANIKHANKAHDANI

April 2012

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Program memajukan Jatropha mase kimi telah direkabentuk dengan objektif utama adalah untuk menghasilkan bahan tanaman yang unggul, yang memberikan hasil biji dan minyak yang tinggi sebagai bahan tanaman komersial. Objektif khusus adalah untuk mengkaji variasi secara intra dan inter-populasi melalui morfologi dan penanda molekul inter ulangan jujukan ringkas (ISSR), untuk menganggar komponen variasi genetik, nilai keterwarisan, keupayaan bergabung am dan khusus bagi beberapa ciri penting bagi mengenalpasti waris induk yang terbaik dan akhirnya untuk menentukan paras heterosis bagi hibrid F_1 di dalam kacukan dialel. Populasi Jatropha dari Malaysia, Indonesia, India dan Filipina telah digunakan sebagai populasi asas di dalam program pemilihan ini.

Enam puluh empat pokok bagi setiap enam populasi Jatropha, iaitu Malaysia1 (My1), Malaysia2 (My2), Indonesia1 (In1), Indonesia2 (In2), India1 (Id1) dan Filipina1 (Ph1) telah ditanam untuk penilaian ladang di Taman Pertanian Universiti Serdang, Universiti

Putra Malaysia. Populasi Jatropha tersebut telah disusun menggunakan rekabentuk blok penuh terawak (RCBD) dengan empat replikasi pada jarak tanaman 4 m × 2 m. Analisa varians bagi ciri biji menunjukkan terdapat variasi yang berbeza di kalangan populasi Jatropha yang dikaji untuk panjang biji, lebar biji, berat biji dan berat 100-biji pada $P \leq 0.05$. Populasi My2 mengeluarkan kandungan minyak yang tertinggi untuk kedua-dua tahun pengeluaran hasil (31.73 dan 33.41%). Kandungan minyak yang terendah adalah didapati di dalam populasi In1 untuk tahun pertama dan kedua penuaian (masing-masing 29.09 dan 30.61%).

Variasi bererti ($P \leq 0.05$) didapati bagi tinggi pokok, bilangan dahan sekunder, bilangan hari berbunga, bilangan jambak bunga dan hasil biji. Hasil biji tertinggi didapati dalam populasi In1 dalam kedua-dua tahun, manakala yang terendah adalah populasi In2 untuk tahun pertama dan My1 untuk tahun kedua penuaian. Hasil biji mempunyai korelasi bererti dengan bilangan hari berbunga, bilangan jambak bunga dan tinggi pokok. Hasil biji juga mempunyai korelasi bererti dengan kandungan minyak. Bilangan hari berbunga didapati mempunyai perhubungan positif bererti dengan tinggi pokok, bilangan dahan sekunder, bilangan dahan tertiari, bilangan jambak bunga, panjang biji dan berat biji. Nilai keterwarisan tertinggi didapati pada berat 100-biji (77.3%). Nilai keterwarisan untuk tinggi pokok, bilangan dahan sekunder, bilangan hari berbunga, berat biji, panjang biji, hasil biji dan kandungan minyak adalah masing-masing 40.8, 47.8, 31.7, 77.2, 52.0 dan 49.7%.

ISSR telah digunakan untuk menentukan variasi inter dan intra-populasi di dalam populasi asas tersebut. Peratusan jalur polimorfik untuk setiap populasi berjulat antara

46.15 hingga 60.84%, dengan purata 55.10%. Di kalangan populasi tersebut bilangan purata alel per lokus (n_a) berjulat dari 1.46 (My1) hingga 1.61 (Ph1) dengan bilangan purata adalah 1.55, bilangan alel efektif per lokus (n_e) berjulat dari 1.12 (My1) hingga 1.17 (My2) dengan bilangan purata adalah 1.15.

Di kalangan populasi tersebut, populasi My1 mempunyai paras kepelbagaian genetik yang terendah, manakala populasi Ph1 menunjukkan kepelbagaian genetik yang tertinggi. Pembahagian kepelbagaian genetik kepada di kalangan dalam populasi dan di antara populasi berdasarkan indeks kepelbagaian Shannon juga membuktikan terdapat lebih variasi di kalangan dalam populasi (0.81) berbanding variasi di antara populasi (0.19).

Dari penilaian di ladang populasi Jatropha tersebut yang terdiri dari 364 pokok, sepuluh individu pokok yang unggul (tiga dari populasi Malaysia, tiga dari Indonesia, dua dari India dan dua dari Filipina) telah dipilih dan dijalankan kacukan dikalangan mereka menggunakan rekabentuk pengawanan separa-dialel (10×10) termasuk penyendirian bagi setiap pokok induk terpilih. Empat puluh lima hybrid F_1 dan 10 progeni penyendirian telah dinilai di ladang mengunakan rekabentuk blok penuh terawak (RCBD) dengan tiga replikasi di Taman Pertanian Universiti Pucong, Universiti Putra Malaysia. Analisa varians menunjukkan terdapat variasi yang bererti ($p \leq 0.01$) dikalangan induk dan hibrid bagi semua ciri. Analisa varians bagi kesan gen menunjukkan keupayaan bergabung am (GCA) dan khusus (SCA) mempengaruhi kesemua ciri. Analisis keupayaan bergabung khusus dalam kajian ini menunjukkan beberapa hibrid menberikan kesan SCA yang bererti untuk setiap ciri dalam individu hibrid yang berlainan tetapi tiada satu hibridpun yang memberikan kesan SCA baik bagi semua ciri dalam sesuatu hibrid tertentu.

Peratusan heterosis untuk ciri dalam sebahagian hibrid adalah agak tinggi menunjukkan satu tahap kepelbagaian genetik yang tinggi dikalangan induk. Keterwarisan luas yang tinggi telah direkodkan untuk semua ciri. Walaubagaimanapun, nisbah GCA/SCA menunjukan pengaruh gen bukan-aditif adalah lebih penting berbanding pengaruh gen aditif. Analisa GCA mendapati tiada satu induk pun mempunyai berkeupayaan bergabung am untuk semua ciri secara serentak.

ACKNOWLEDGEMENT

My special and sincere appreciation goes to my supervisor of this thesis, Assoc. Prof. Dr. Mohd Rafii Bin Yusop. I am extremely appreciative of his supervision, kindness, constant support and endless patience. Without his well-designed plan and careful review of the draft, this thesis would have never been possible. His kind encouragement, guidance and instruction are invaluable things for me forever. I wish to express my gratitude to my thesis co-supervisors Prof. Dr. Ghizan Saleh and Prof. Dr. Zakaria Wahab for their guidance.

Many thanks are expressed to all teachers and staff of Crop Science Department, Universiti Putra Malaysia, for their help in many ways while I was studying in Malaysia. Special thanks go to Assist. Prof. Dr. Sasan Ghasemi for his encouragement. I would like to thank Mr. Mahmoodreza Shabanimofrad, Mr. Ali Ranjbarfard and Ms Mozhdeh Ebrahimpour and all other Iranian and Malaysian friends here for their help.

My greatest appreciation goes to my sister, Roya Biabani for her kindness and taking care of me during stay in Malaysia. Finally, I would like to express my special thanks to my parents and my sisters for their encouragement, support and understanding, without which the study would have not been successfully completed.

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I certify that a Thesis Examination Committee has met on ….. to conduct the final examination of Alireza Biabanikhankahdani on his thesis entitled "Genetic and molecular analyses for seed and oil yields improvement in *Jatropha curcas* L. populations" in accordance with the Universities and Univesity Colleges Act 1971 and the Constitution of the Pertanian Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement of the Doctor of Philosophy of Agricultural Science. The members of the Supervisory Committee were as follows:

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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, or is not, concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

ALIREZA BIABANIKHANKAHDANI

Date: 24 April 2012

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
bp	Base pairs
h^2_B	Broad sense heritability
CTAB	Cetyltrimethylammonium bromide
G_{ST}	Coefficient of gene differentiation
CV	Coefficient of variation
r	Correlation coefficient
DNA	Deoxyribonucleic acid
n_e	Effective number of alleles
Nm	Estimate of gene flow
FAO	Food and Agriculture Organization of the United Nations
H_S	Gene diversity within populations
GA	Genetic advance
GCV	Genotypic coefficient of variation
GV	Genotypic variance
ha	Hectare
ISSR	Inter simple sequence repeat
IPGRI	International Plant Genetic Resource Institute
MS_E	Mean square of error

MS_G	Mean square of genotype
masl	Metre above sea level
h^2_B	Narrow sense heritability
h	Nei's (1973) gene diversity
N	North
NPB	Number of polymorphism bands
NTSYS	Numerical taxonomy multivariate analysis system
n_a	Observed number of alleles
ppm	Parts per million
PPB	Percent of polymorphic bands
PCV	Phenotypic coefficient of variation
PV	Phenotypic variance
PCR	Polymerase chain reaction
PC	Principal component
PCA	Principle component analysis
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
rpm	Revolution per minute
I	Shannon's information index
SSR	Simple sequence repeat
SE	Standard error
Taq	Thermus aquaticus

H_t	Total gene diversity
TE	Tris EDTA buffer
TBE	Tris-borate/EDTA
UV	Ultraviolet
UPGMA	Unweighted pair group method using arithmetic averages

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The employment of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. Biodiesel, an alternative fossil fuel, is produced from renewable biological sources such as vegetable oils and animal fats (Fangrui and Milford, 1999).

A hundred years ago, Rudolf Diesel tried vegetable oil as fuel for his engine (Shay, 1993). In the 1930s and 1940s vegetable oils were utilized as diesel fuels from time to time, but usually only in emergency conditions. In recent times, because of increase in crude oil prices, limited resources of fossil oil and environmental concerns there has been a renewed focus on vegetable oils to make biodiesel fuels (Shay, 1993; Fangrui and Milford, 1999). Recently, biofuels extracted from plant species such as *Jatropha curcas* L. has been a major renewable source of energy (Mukherjee *et al.*, 2011).

Biomass as a source of renewable energy is essential for the development and sustenance of civilization. Because of increasing attention in renewable energy sources, liquid bio-energy production from vegetable oils has been seen as one of the possible options to decrease greenhouse gas emissions (Mukherjee *et al.*, 2011). There is a growing interest in technologies to produce biofuels and make them cost competitive with fossil petroleum extracted fuels. Global consciousness of the climatic changes linked to a gradual increase in CO_2 emission in the atmosphere is producing a relative impact on the societies of developed and developing countries (Fangrui and Milford, 1999).

Regarding the abundant benefits of this technology, many countries including Malaysia are interested to invest in it. During 2004–2005, the biodiesel production from vegetable oils was appraised at 2.36 million tonnes globally. Of this, EU and USA accounted for about 82 and 6%, respectively. Global biodiesel production is set to reach some 24 billion liters by 2017 (Divakara *et al.*, 2010).

Jatropha is one of the most promising biofuel sources today. About 30% of the Jatropha seed is comprised of oil. This oil can be easily processed into fuel that can replace or mixed with petroleum based diesel to save on imported oil and most importantly to raise the utilization of green energy (Openshaw, 2000). During the recent years, *J. curcas* has received much attention as a good substitute for fossil fuel in Malaysia. Since Jatropha is a species which can be cultivated by farmers on marginal agricultural farms, it is very logical to invest in Jatropha besides the palm industry.

Regarding large semi wild distribution of Jatropha in different parts of Malaysia, it would be expected to have substantial genetic variation. Environmental factors in addition togenetic and physiological factors play important role in determination of plant potential for seed quality. These characters seem to be under strong genetic control (Ginwal *et al*., 2005). Depending on the species, germination responses of seed alter in accord with geographical and environmental factors, for example: latitude, elevation, soil nutrient, soil moisture, temperature, density and kind of plant cover (Heller, 1996; Ginwal *et al.*, 2004).

Substantial genetic variation in growth, chemical composition of seed and seed traits at the level of provenance, variety, accession and progeny can be assumed specifically in out crossing species such as many species of Jatropha, *Acacia* and *Prosopis*, which are widely employed in agro-forestry systems overall the world wide landscapes. The variation would be practical as a source for future genetic selection of desired ideo types (Von Carlowitz, 1986). Heavy and bold seeds make possible the seedling to grow vigorously (Kaushik *et al.*, 2003) and better seedling vigor will to lower nursery management time and the maintenance cost. Genetic variation in seed morphology and oil content of Jatropha can be of extensive potential in plant improvement programs, especially selection of genotypes having more seed yield and oil content (Kaushik *et al.*, 2003).

The screening of existing variation in populations for growth and oil yield could be utilized profitably for selection of best genotypes for production of oil. There is a good reason for developing Jatropha as a new energy crop as it does not compete with conventional food crops for land, water and manpower resources and also it has the ability to make a significant contribution to the nation's growing needs for energy though large scale cultivation with ease (Ginwal *et al*., 2004) .

The superior genotype in a plant breeding program is with high yielding, fastest growing and most resistant to diseases and pests. There is no genotype, which has all these characteristics (Gadow and Bredenkamp, 1992). The superior genotype always represents stability. It performs better than other plant when all the criteria together are considered; anyway it may be out performed in any one of the criteria. However, in case of Jatropha, number of branches, number of inflorescences, number of fruits, number of seeds and oil content may be more important traits compared to other traits. The superior plant selection is the first and most important criteria in any plant improvement program. The selection of the superior genotype is based upon various important traits associated with the species and their relative ranking (Mishra, 2009). Genetic diversity underlies the improvement of crops in plant breeding (Pervaiz *et al.*, 2010) and plant breeders lean on the availability of genetic diversity during selection in cultivar development (Ahmed *et al.*, 2010).

Evaluation of the genetic diversity can be computed from utilizing molecular markers (Ahmed *et al.*, 2010). Recently, PCR-based methods for analyzing diversity of the collected populations are usual procedures for revealing and classifying the populations. Molecular marker methods are supporting the classic methods such as physiological and morphological characteristics (Solouki *et al.*, 2008) but the advantage of these techniques is their capacity to discover genetic diversity a more powerful level of resolution than other methods (Sergio and Gianni, 2005).

Therefore, development of diversity studies with molecular genetic analysis for the available populations is significant for Jatropha improvement. Several DNA marker techniques are available to study genetic diversity. But among these, ISSR is an inexpensive and rapid method not requiring any information regarding the genome of the plant, and has been widely used to ascertain the genetic diversity in several plants (Ganesh Ram *et al.,* 2008). In the present study, ISSR markers were utilized to investigate the level and distribution of genetic diversity in different population of Jatropha.

Several researchers have reported crossing and intensive selection between species with desirable traits of Jatropha. Although hybrids have been made in Jatropha diallel mating were not employed to determine general and specific combining ability, heterosis and compatibility. Stability of varieties has not been assayed over different environments.

Therefore, genetic diversity studies using molecular markers (ISSR), morphological markers, seed yield and oil content are important to determine genetic diversity in *Jatropha curcas*. In combination with diallel crosses, heterotic groups and performances can be estimated and stable varieties recognized. Molecular marker knowledge will increase efficiency and effectiveness of marker assisted breeding and in conservation of plant genetic resources. Improved Jatropha varieties are urgently needed to improve the Jatropha market through seed yield and oil content as these factors affect oil price on the world market.

1.2 Statement of problem

Jatropha is a new crop for Malaysia but the government and private sector is very highly interested in Jatropha oil as a new source of biodiesel. However there is no planting material (seeds) produced locally and they are importing seeds from abroad. In addition, there is lack of published information with regards to genetics and breeding and agronomic performance of Jatropha in Malaysia. It is important to produce local varieties with genetic superiority in yield. From this study, comprehensive information on genetics and agronomic performance of Jatropha evaluated in Malaysia will be published.

1.3 Research objectives

The present study was conducted with the following objectives

Main objective

To identify and select parent lines with high seed and oil yields for commercial planting

Specific objectives

1. To determine inter and intra-population variation using morphological and ISSR molecular markers.
2. To estimate genetic components and heritability values of the more important characters in the Jatropha populations.
3. To estimate general and specific combining abilities for identifying the best parental lines and the best hybrids.
4. To quantify the level of heterosis for F_1 hybrids in diallel crosses.

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