



**UNIVERSITI PUTRA MALAYSIA**

***PERFORMANCE OF KENAF (*Hibiscus cannabinus* L.)  
GENOTYPES AND THEIR GENETIC VARIABILITY BASED  
ON DNA MICROSATELLITE MARKERS***

**MAJID FOROUGH**

**FP 2012 27**

**PERFORMANCE OF KENAF (*Hibiscus cannabinus* L.) GENOTYPES AND  
THEIR GENETIC VARIABILITY BASED ON DNA MICROSATELLITE  
MARKERS**

By

**MAJID FOROUGHI**

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

**June 2012**

## DEDICATIONS

*Dedicated with Love to*

*My Kind Father, Mohammad Ali Foroughi*

*and*

*My Beloved Mother, Khorshid Abroi*

*For Their Endless Love, Support and Sacrifices*



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

**PERFORMANCE OF KENAF (*Hibiscus cannabinus* L.) GENOTYPES AND THEIR GENETIC VARIABILITY BASED ON DNA MICROSATELLITE MARKERS**

By

**MAJID FOROUGHJI**

**June 2012**

**Chairman: Prof. Ghizan Bin Saleh, PhD**

**Faculty: Agriculture**

Kenaf (*Hibiscus cannabinus* L.) is an annual fiber crop of the Malvaceae family and originated from the tropics of east and central Africa. It is a multipurpose crop for making pulp and paper, and biocomposites. A study was conducted to compare morphological and agronomic performances of 40 kenaf accessions grown on mineral soil in Serdang, Selangor and BRIS soil in Bachok, Kelantan Malaysia, to estimate heritability of important agronomic traits, to determine phenotypic correlations among the agronomic traits measured on those accessions, and to assess genetic diversity among the accessions using microsatellite (SSR) DNA markers.

In general, all the kenaf accessions performed better on mineral soil than they did on BRIS soil. Accession CQ3205 was found to be the best performing accession on mineral soil, but however, it did not perform significantly better than the control variety, V36.

Among the accessions evaluated on BRIS soil, IX51 was found to produce the highest fresh plant yield, fresh stalk yield, dry stalk yield, dry bast yield and dry core yield, which were significantly better than those of the control variety, V36. However, many accessions were found to have out-yielded the control variety on BRIS soil. Results of the analysis of variance showed significant genotype by location interaction for all traits measured, indicating severe influence of environmental factors, particularly the soil conditions, on performance of the accessions, for yield and its components at the locations. This has resulted in many cases high-yielding accessions in Serdang performed poorly in Bachok and *vice versa*.

Investigation of genetic diversity among the 40 kenaf accessions using four morphological and 16 agronomic traits revealed a wide range of variation. Results of ANOVA also showed significant variation for all the agronomic traits measured.

Genetic diversity among the accessions was also investigated using 10 microsatellite (SSR) markers. Results showed that SSRs were informative molecular markers for detecting genetic differences among kenaf genotypes, as indicated by the high Nei's gene diversity coefficient and polymorphic information content (0.55 and 0.50, respectively). Among the SSR primers amplified, Ht-18 and Ht-40 were found to be the most informative SSR markers to exhibit genetic variation among the accessions (with PIC values of 0.77 and 0.74, respectively).

Genetic similarity among the accessions obtained from amplification of the SSR markers was found to be low. The highest genetic similarity (0.73) was found between Accessions 15 and 7-1X, indicating similarity in the loci they possessed. In contrast, the SSR primers used revealed high level of dissimilarity (similarity coefficient of 0.00) between seven pairs of accessions *viz.*, Ghana 07 and El Salvador, Cuba 797 and A63-478, G7 and El Salvador, Mahmur and A63-478, Mahmur and Everglade 71, G7 and Guatemala 4, and G7 and BG53-42. This indicates high genetic diversity between these pairs of accessions as they possessed dissimilar alleles at all the loci amplified.

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

**PRESTASI GENOTIP KENAF (*Hibiscus cannabinus* L.) DAN KEPELBAGAIAN GENETIKNYA BERDASARKAN PENANDA DNA MIKROSATELIT**

Oleh

**MAJID FOROUGHJI**

**Jun 2012**

**Pengerusi: Prof. Ghizan Bin Saleh, PhD**

**Fakulti: Pertanian**

Kenaf (*Hibiscus cannabinus* L.) adalah tanaman fiber semusim dari famili Malvaceae dan berasal daripada kawasan tropika di Afrika timur dan tengah. Ia adalah tanaman seberguna yang digunakan untuk pembuatan pulpa dan kertas, dan biokomposit. Satu kajian telah dijalankan untuk membandingkan prestasi morfologi dan agronomi 40 aksesori kenaf yang ditanam di tanah mineral di Serdang, Selangor dan di tanah BRIS di Bachok, Kelantan Malaysia, untuk menganggarkan kebolehwarisan sifat-sifat agronomi penting, dan menentukan korelasi fenotip di antara sifat-sifat yang diukur pada aksesori tersebut, dan menilai kepelbagaian genetik antara aksesori menggunakan penanda DNA mikrosatelit (SSR).

Secara amnya, kesemua aksesori kenaf tersebut menunjukkan prestasi yang baik di tanah mineral berbanding yang ditanam di tanah BRIS. Aksesori CQ3205 dikenalpasti sebagai aksesori terbaik di tanah mineral namun prestasinya tidak melebihi varieti kawalan, V36 secara signifikan. Di tanah BRIS, IX51 didapati menghasilkan hasil pokok segar, hasil batang segar, hasil batang kering, hasil kulit kering dan hasil teras kering yang tertinggi di antara semua aksesori yang diuji, yang mana ianya lebih baik daripada varieti kawalan, V36 secara signifikan. Walau bagaimanapun, banyak aksesori yang diuji di tanah BRIS adalah lebih baik daripada varieti kawalan. Keputusan analisis varians menunjukkan interaksi antara genotip dan persekitaran yang signifikan bagi semua sifat yang diukur, membuktikan pengaruh faktor persekitaran yang kuat, khususnya keadaan tanah, terhadap prestasi aksesori, untuk hasil dan komponen-komponen hasil di lokasi. Ini telah menyebabkan dalam banyak keadaan, aksesori yang tinggi hasilnya di Serdang menunjukkan prestasi yang rendah di Bachok dan sebaliknya.

Penelitian kepelbagaian genetik di kalangan 40 aksesori kenaf menggunakan empat sifat morfologi dan 16 sifat agronomi menunjukkan julat variasi yang besar. Keputusan dari ANOVA telah menunjukkan variasi yang signifikan untuk semua sifat-sifat agronomi yang diukur.

Kepelbagaian genetik antara aksesori juga diteliti menggunakan 10 penanda mikrosatelit (SSR). Keputusan menunjukkan bahawa penanda SSR adalah penanda molekul yang informatif di dalam mengenalpasti perbezaan dari sudut genetik di antara aksesori kenaf, sebagaimana ditunjukkan oleh nilai pekali kepelbagaian gen Nei dan nilai kandungan



informasi polimorfik yang tinggi (masing-masing, 0.55 dan 0.5). Di antara penanda molekul SSR yang diamplifikasi, Ht-18 dan Ht-40 didapati sebagai penanda SSR yang paling informatif untuk menunjukkan variasi genetik antara aksesori (dengan nilai kandungan informasi polimorfik masing-masing 0.77 dan 0.74).

Kesamaan genetik antara aksesori yang diperolehi dari amplifikasi penanda SSR adalah rendah. Kesamaan genetik yang tertinggi (0.73) didapati di antara aksesori 17 dan aksesori 7-1X, menunjukkan kesamaan pada lokus-lokus yang dimiliki. Sebaliknya, primer SSR tersebut menunjukkan tahap ketidaksamaan yang tinggi (pekali persamaan 0.00) di antara tujuh pasangan aksesori berikut: Ghana 07 dan El Salvador, Cuba 797 dan A63-478, G7 dan El Salvador, Mahmur dan A63-478, Mahmur dan Everglade 71, G7 dan Guatemala 4, dan G7 dan BG53-42. Ini membuktikan kepelbagaian genetik yang tinggi antara pasangan-pasangan aksesori yang disebutkan, kerana ia memperoleh alel-alel yang berlainan pada semua lokus yang diamplifikasi.

## ACKNOWLEDGEMENTS

All praises and thanks are due to Allah Almighty for His Mercy and Grace.

I would like to express my sincere thanks to Professor Dr. Ghizan Bin Saleh, chairman of my supervisory committee, for his dedicated efforts, support, invaluable advice and intellectual guidance during the accomplishment of this research work. I would also like to thank my supervisory committee members, Associate Professor Dr. Jalaluddin Harun and Dr. Nur Ashikin Psyquay Abdullah for their guidance, assistance, encouragements and constructive comments throughout the period of this study. I greatly appreciate all the help and support provided by the supervisory committee during my study in Malaysia.

Special thanks go to the Ministry of Higher Education (MOHE) Malaysia for the financial support of the research under the Fundamental Research Grant Scheme (FRGS) Top Down, Project Phase 1/2007, through Professor Dr. Ghizan Bin Saleh. Many thanks and appreciations also go to Universiti Putra Malaysia (UPM) for providing me the financial support under the Special Graduate Research Allowance (S-GRA) through the kind support of Professor Dr. Ghizan Bin Saleh.

I am indebted to Dr. Sally Dillon, Research Scientist of the Australian Tropical Crops and Forages Germplasm Collection for providing kenaf accessions for my study, Mr. A. Khamil Hj. Mustapha from the National Kenaf and Tobacco Board (LKTN) for the help

rendered during evaluation of the kenaf accessions in Bachok, Kelantan, and Mr. Mohd. Ghazali Mohd. Satar, Senior Agriculture Officer of the Faculty of Agriculture, Universiti Putra Malaysia for his assistance during my study.

I am very grateful to Mrs. Siti Nadirah Dasar and all staff/officers of Field 10, Universiti Putra Malaysia for their help during my field work. I am also grateful to the laboratory technicians of the Department of Crop Science, Universiti Putra Malaysia. The help and assistance provided by Mr. Rosdi Abd Ghani and Mr. Mohd Helmy Hamisan are highly appreciated.

My sincere thanks and appreciations also go to my colleagues and fellow students especially Mrs. Zahra Noori and Mr. Rahmatollah Behmaram in the Plant Breeding Laboratory, Crop Science Department, Universiti Putra Malaysia for their help, support and encouragements. Special thanks go to my dearest friend, Mr. Pedram Kashiani, for his kindness, co-operation and involvements in discussions during the study. Acknowledgements are also extended to all my friends at Universiti Putra Malaysia, for their warm friendship and constant encouragements during the period of my study.

My deepest gratitude goes to my father Mohammad Ali and my mother Khorshid for their help and continuous moral support throughout my study. The help and encouragements provided by my sister, Homa, and my brothers Ahmad Reza, Ali Reza and Saeid are greatly appreciated.

I certify that a Thesis Examination Committee has met on 15 June 2012 to conduct the final examination of Majid Foroughi on his thesis entitled “Performance of kenaf (*Hibiscus cannabinus* L.) genotypes and their genetic variability based on DNA microsatellite markers” in accordance with the Universities and University Colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

**Mihdzar Bin Abdul Kadir, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Mohd Rafii Bin Yusop, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Faridah Binti Qamaruz Zaman, PhD**

Associate Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Mohamad Osman, PhD**

Professor  
Faculty of Science  
International Islamic University Malaysia  
(External Examiner)

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 27 August 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Ghizan Bin Saleh, PhD**

Professor  
Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Jalaluddin Harun, PhD**

Associate Professor  
Institute of Tropical Forestry and Forest Products  
Universiti Putra Malaysia  
(Member)

**Nur Ashikin Psyquay Abdullah, PhD**

Senior Lecturer  
Agriculture  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## 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 institutions.



---

**MAJID FOROUGHI**

Date: 15 June 2012



## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	ix
<b>APPROVAL</b>	xi
<b>DECLARATION</b>	xiii
<b>LIST OF TABLES</b>	xvii
<b>LIST OF FIGURES</b>	xix
<b>LIST OF ABBREVIATIONS</b>	xxi
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>5</b>
2.1 Origin of Kenaf	5
2.2 Taxonomy and Botany of Kenaf	5
2.3 Importance of Kenaf	7
2.3.1 Production of Kenaf	7
2.3.2 Uses of Kenaf	8
2.4 Agronomy of Kenaf	9
2.4.1 Climate and Adaptability	9
2.4.2 Varieties	10
2.4.3 Seedbed Preparation and Fertilization	11
2.4.4 Plant Population and Row Spacing	12
2.4.5 Harvest Time	13
2.5 Mineal and BRIS soils in Malaysia	13
2.6 Kenaf Fiber Morphology and Quality	14
2.7 Germplasm Collection	15
2.8 Genotype by Environment Interaction	16
2.9 Heritability	18
2.10 Phenotypic Correlations Among Agronomic Traits	19
2.11 Visualization of the Relationship between Genotypes and Traits	20
2.12 Genetic Diversity Assessment Methods	21
2.12.1 Morphological and Agronomic Characterization	22
2.12.2 Molecular Markers	23
2.12.2.1 Restriction Fragment Length Polymorphism (RFLP)	24

	2.12.2.2	Random Amplified Polymorphic DNA (RAPD)	24
	2.12.2.3	Amplified Fragment Length Polymorphism (AFLP)	25
	2.12.2.4	Simple Sequence Repeats (SSR)	27
<b>3</b>		<b>EVALUATION OF AGRONOMIC PERFORMANCE OF KENAF GENOTYPES ON MINERAL AND BRIS SOILS IN MALAYSIA</b>	<b>30</b>
3.1		Introduction	30
3.2		Materials and Methods	32
	3.2.1	Plant Materials	32
	3.2.2	Locations of Experiment	32
	3.2.3	Experimental Design and Cultural Practices	34
	3.2.4	Data Collection	35
	3.2.5	Data Analysis	41
	3.2.5.1	Analysis of Variance	41
	3.2.5.2	Test for Homogeneity of Error Variances	41
	3.2.5.3	Combined Analysis of Variance across Locations	42
	3.2.5.4	Broad-sense Heritability ( $h_B^2$ ) for Traits	43
	3.2.5.5	Phenotypic Correlations Among Traits	45
	3.2.5.6	Association of Traits with Specific Accessions	45
3.3		Results	47
	3.3.1	Variation Among Accessions based on Morphological Traits	47
	3.3.2	Results of Analysis of Variance for Traits Measured on Accessions in Serdang and Bachok	54
	3.3.3	Results of Analysis of Variance for Traits Measured on Accessions at the Two Locations Combined	57
	3.3.4	Performance of Accessions	59
	3.3.5	Broad-sense Heritability ( $h_B^2$ ) for Traits Measured on Accessions Evaluated in Serdang, Bachok and at the Two Locations Combined	74
	3.3.6	Phenotypic Correlations Among Traits Measured in Serdang and Bachok	76
	3.3.7	Association of Agronomic Traits with Accessions	84
3.4		Discussion	90
<b>4</b>		<b>ASSESSMENT OF GENETIC DIVERSITY IN KENAF USING SSR MARKERS</b>	<b>98</b>
4.1		Introduction	98
4.2		Materials and Methods	100



4.2.1	Plant Materials	100
4.2.2	DNA Extraction	101
4.2.3	DNA Quantification	102
4.2.4	PCR Reaction	103
4.2.5	Metaphor Gel Electrophoresis	103
4.2.6	Data Analysis	105
4.3	Results	107
4.3.1	Microsatellite Marker Efficiency in Detecting Genetic Variation Among Accessions	107
4.3.2	Genetic Differentiation Among and Within Accessions Revealed by SSR Markers	110
4.3.3	Genetic Diversity Among Accessions Revealed by SSR Markers	113
4.4	Discussion	120
<b>5</b>	<b>GENERAL DISCUSSION AND CONCLUSION</b>	<b>125</b>
	<b>REFERENCES</b>	<b>129</b>
	<b>APPENDICES</b>	<b>142</b>
	<b>BIODATA OF STUDENT</b>	<b>152</b>