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PERFORMANCE AND GENETICS OF KENAF (Hibiscus cannabinus L.) FOR FORAGE YIELD AND QUALITY

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PERFORMANCE AND GENETICS OF KENAF (*Hibiscus cannabinus* L.) FOR FORAGE YIELD AND QUALITY

By

ZAHRA NOORI

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

November 2014

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DEDICATION

Dedicated to

My beloved **parents** who are the greatest source of inspiration in my life,

My beloved husband, **Majid**, for his support and patience,

My **brothers** and **sisters** for their constant support and encouragement,

and

My little angel **Aria** for making my life more meaningful.

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

PERFORMANCE AND GENETICS OF KENAF (*Hibiscus cannabinus* L.) FOR FORAGE YIELD AND QUALITY

By

ZAHRA NOORI

November 2014

Chairman: Professor Ghizan Bin Saleh, PhD

Faculty: Agriculture

Genetic studies on kenaf (*Hibiscus cannabinus* L.), a potential forage crop, is necessary to improve kenaf varieties for consumption as a ruminant feed. The main objectives of the present study were to evaluate selected kenaf varieties for forage yield and quality, estimate heritability, heterosis, and genetic parameters involved in the control of forage yield and quality in kenaf populations using generation means analysis, and identify genetic factors associated with kenaf forage yield and quality using SSR markers.

Forty kenaf accessions were used to evaluate forage yield and quality. The variations among kenaf accessions were significant for most of the traits measured, except stem-ADF. Accession IX51 was found to have the highest fresh plant yield. IX51 was also found to produce the highest plant dry matter yield followed by G46, which were significantly higher than that of the control variety, V36. Accession 15 produced highest fresh leaf yield, while IX51 showed the highest leaf DM yield. Accessions 75-71, Everglade 41, KK60 and Tainung 1 were found to have with high CP content. Accessions with high plant yields and CP content were used as parents for further evaluation. Further evaluation was conducted on eight selected parental varieties with V36 as the control variety. Accession Cuba 2032 with high plant dry matter yield and leaf dry matter yield and Accessions 75-71 and Everglade 41 with high CP content were selected for further evaluation in a subsequent experiment. Based on the evaluation of performance of the kenaf accessions, 1X51 and Cuba 2032 with highest yields and Accessions 75-71 and Everglade 41 with the highest CP content were selected as parents. These accessions were significantly better than the control variety, V36 and could be used for animal production. Results of correlations indicated that plant yield was positively correlated with yield related components, while it was negatively correlated with leaf to stem ratio.

When generation of a population revealed significant differences in a trait, analysis of generation means was employed to study genetics of the trait. The effects of basic generations were significant for all the traits measured in Cross 1

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(Cuba 2032 × Accession 75-71) and Cross 2 (1X51 × Everglade 41). F_1 mean performances in both crosses were higher than mid-parents values for all traits measured, except days to flowering. Results revealed that both additive and non-additive effects were important in the inheritance of the traits measured in the two crosses, indicating that some of the traits were not simply inherited. Thus, selection could be carried out in later segregating generations. The additive gene effects had a higher contribution than dominant gene effects for most of the traits measured in Cross 1, while dominant gene effects had greater contribution than additive gene effects for some traits measured in Cross 2. High broad-sense heritability for all the traits and high to moderate narrow-sense heritability for most of the traits, except leaf-ADF and stem-ADF, were found in Cross 1. High heritability implies a strong resemblance between parents and offspring with regards to a specific trait. However, in Cross 2, high broad-sense heritability for most of the traits and low to moderate narrow-sense heritability was found for all the traits measured. Positive heterosis for all the traits measured, except for days to flowering, indicates the importance of hybrid vigor for the traits in the two crosses. Negative heterosis for days to flowering, indicates that the hybrid flowered earlier than parents which is good for quality.

One hundred and fifty individual F_2 plants obtained from the Cuba 2032 × Accession 75-71 cross were used for marker-trait association and to construct a primary genetic linkage map using simple sequence repeats (SSRs). Among SSR markers, five SSRs were found to be closely associated with plant height, leaf to stem ratio, leaf-CP content, stem-CP content, and stem-ADF. Seven polymorphic SSR markers were also randomly distributed on a linkage group with a covering of 128.2 cM. The construction of the kenaf genetic linkage group will be useful for further genetic studies including mapping both qualitative and quantitative traits, marker-assisted selection programs, and comparative genomic analysis.

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PRESTASI DAN GENETIK BAGI KENAF (*Hibiscus cannabinus* L.) UNTUK HASIL DAN KUALITI FORAJ

Oleh

ZAHRA NOORI

November 2014

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Fakulti: Pertanian

Kajian genetik ke atas kenaf (*Hibiscus cannabinus* L.), sebagai tanaman foraj yang berpotensi, adalah perlu untuk memperbaiki varieti kenaf untuk kegunaan makanan haiwan ruminan. Kenaf adalah tanaman herba semusim daripada famili Malvaceae. Semasa peringkat tumbesaran awal, tumbuhan ini mempunyai kandungan protein yang tinggi, membuatkan ia sesuai untuk dijadikan foraj untuk haiwan ternakan. Objektif utama kajian ini adalah untuk menilai aksesi kenaf untuk hasil dan kualiti foraj, untuk menganggar nilai kebolehwarisan, heterosis dan parameter-parameter genetik bagi populasi kenaf dengan menggunakan Analisis Nilai Tengah Generasi (GMA), untuk memperbaiki kaedah kacukan kenaf, dan untuk mengenalpasti faktor genetik yang berkaitan dengan hasil dan kualiti kenaf dengan menggunakan penanda molekul SSR. Empat puluh aksesi kenaf telah digunakan untuk menilai hasil dan kualiti foraj. Kepelbagaian di kalangan aksesi kenaf adalah bererti untuk kesemua sifat yang dikaji, kecuali bagi diameter batang dan kandungan asid “detergent fiber” (ADF) untuk batang. Nilai hasil pokok segar adalah yang tertinggi bagi aksesi IX51, diikuti dengan G46 dan Cuba 2032. 1X51 juga didapati menghasilkan hasil pokok kering yang tertinggi diikuti oleh G46, di mana kedua-duanya adalah lebih bererti daripada varieti kawalan, V36. Aksesi 15 menghasilkan hasil daun segar yang paling tinggi, manakala IX51 menunjukkan hasil daun kering yang paling tinggi. Aksesi yang dikenalpasti mempunyai hasil tinggi digunakan sebagai induk untuk kajian selanjutnya. Aksesi 75-71, Everglade 41, KK60 dan Tainung 1 dengan kandungan CP yang tinggi juga dipilih sebagai induk. Penilaian seterusnya dilakukan ke atas lapan aksesi terpilih dengan V36 sebagai varieti kawalan. Aksesi Cuba 2032 dengan hasil pokok kering dan hasil daun kering yang tinggi dan aksesi 75-71 dan Everglade 41 yang mempunyai nilai kandungan CP yang tinggi telah dipilih untuk penilaian selanjutnya dalam eksperimen seterusnya. Berdasarkan keputusan daripada penilaian prestasi aksesi kenaf, 1X51 dan Cuba 2032 dengan hasil yang tertinggi dan aksesi 75-71 dan Everglade 41 dengan kandungan CP yang tertinggi telah dipilih sebagai induk. Aksesi-aksesi ini menunjukkan nilai yang lebih tinggi daripada varieti kawalan, V36 dan sesuai digunakan untuk penghasilan makanan haiwan. Hasil keputusan kolerasi menunjukkan kolerasi positif yang bererti di

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antara hasil pokok dengan komponen-komponen berkait dengan hasil, serta korelasi yang negatif dengan nisbah daun kepada batang.

Parameter-parameter genetik dianggarkan daripada generasi-generasi asas (P_1, P_2, F_1, F_2, BC_1P_1 dan BC_1P_2) yang diperolehi daripada kacukan Cuba 2032 × aksesi 75-71 dan kacukan 1X51 × Everglade 41 dan ditanam secara berasingan. Kesan daripada generasi-generasi asas adalah bererti diperhatikan untuk kesemua sifat yang dikaji untuk kedua-dua kacukan. Nilai purata generasi F_1 adalah lebih tinggi daripada nilai purata induk untuk kesemua sifat yang diukur, kecuali untuk hari berbunga. Keputusan daripada kedua-dua kesan aditif dan tak-aditif adalah penting untuk pewarisan sifat-sifat yang dikaji untuk kedua-dua kacukan, menandakan yang sebahagian sifat ini tidak diwarisi dengan mudah. Oleh itu, pemilihan harus dijalankan dalam generasi bersegregasi selanjutnya. Kesan gen aditif mempunyai sumbangan yang tinggi berbanding kesan gen dominan bagi kebanyakan sifat yang dikaji untuk Kacukan 1, manakala, kesan gen dominan mempunyai sumbangan yang lebih tinggi berbanding kesan gen aditif untuk kebanyakan sifat yang dikaji dalam Kacukan 2. Kolerasi positif yang tinggi di antara hasil pokok dengan komponen berkait dengan hasil bagi generasi F_2 dalam kedua-dua kacukan menunjukkan pemilihan untuk komponen-komponen berkait dengan hasil ini menyebabkan peningkatan dalam hasil pokok. Tambahan pula, frekuensi taburan individu-individu F_2 untuk semua sifat yang dikaji adalah hampir normal dan menunjukkan kehadiran perwarisan kuantitatif.

Nilai kebolehwarisan luas didapati tinggi untuk kebanyakan sifat yang dikaji dan nilai yang tinggi dan sederhana pula didapati untuk nilai kebolehwarisan sempit untuk semua sifat kecuali ADF daun dan ADF batang untuk Kacukan 1. Kebolehwarisan yang tinggi menunjukkan bahawa sifat-sifat ini mempunyai persamaan yang kuat di antara induk dan anak berdasarkan kepada sifat yang spesifik. Sebaliknya, untuk Kacukan 2, nilai kebolehwarisan luas untuk kebanyakan sifat adalah tinggi dan nilai kebolehwarisan sempit yang rendah dan sederhana ditemui ke atas sifat-sifat yang dikaji. Nilai heterosis yang positif diperoleh untuk semua sifat yang dikaji, kecuali sifat bilangan hari berbunga menunjukkan kepentingan kecergasan hybrid untuk sifat-sifat dalam kedua-dua kacukan. Heterosis yang negatif untuk bilangan hari berbunga, menandakan bahawa hibrid berbunga lebih awal daripada induk di mana ia adalah bagus untuk kualiti. Sebanyak 150 individu dari generasi F_2 yang diambil dari kacukan Cuba 2032 × Aksesi 75-71 telah digunakan untuk membina peta rangkaian genetik primer dengan menggunakan penanda mikrosatelit DNA Simple Sequence Repeat (SSR). Di kalangan penanda SSR, keputusan menunjukkan lima penanda SSR sangat berkait dengan ketinggian pokok, nisbah daun kepada batang, kandungan CP dalam daun, kandungan CP dalam batang, dan ADF batang. Tujuh penanda SSR yang polimorfik tersebar secara rawak di kumpulan rangkaian genom kenaf yang meliputi 128.2 cM. Pembinaan kumpulan rangkaian genetik kenaf ini amat berguna untuk kajian genetik selanjutnya termasuklah pemetaan sifat-sifat kualitatif dan kuantitatif, program pemilihan berasaskan penanda molekul dan analisis perbandingan genom.

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I certify that a Thesis Examination Committee has met on 28 November 2014 to conduct the final examination of Zahra Noori on her thesis entitled "Performance and Genetics of Kenaf (*Hibiscus cannabinus* L.) for Forage Yield and Quality" 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 Doctor of Philosophy.

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

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
AFLP	Amplified fragment length polymorphism
CP	Crude protein
CTAB	Cetyltrimethylammonium bromide
DM	Dry matter
EDTA	Ethylenediamine tetra-acetic acid
GLM	Ground magnesium limestone
HPH	High-parent heterosis
ID	Inbreeding depression
MAS	Marker assisted selection
MPH	Mid-parent heterosis
NDF	Neutral detergent fiber
NFC	Non fiber carbohydrates
NIRS	Near infrared reflectance spectroscopy
NSC	Non structural carbohydrates
OPF	Oil palm fronds
PCA	Principal component analysis
PCR	Polymerase chain reaction
PKC	Palm kernel cake
PVP	Polyvinyl pyrrolidone
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
TBE	Tris-borate/EDTA
TE	Tris EDTA buffer

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CHAPTER 1

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.), which belongs to the Malvaceae family, is an annual herbaceous crop native to central Africa. Kenaf has a wider range of adaptation to climatic and soil conditions compared to other fiber plant species grown for commercial use. It is grown in many countries for fiber, but found potential as a source of feed for ruminant animals (Swingle *et al.*, 1978; Webber *et al.*, 2002; Webber and Bledsoe, 2002).

The various components of the kenaf plants which include the stems, leaves and seeds are found to contain usable portions within each of them, like fibers and fiber strands in stems, protein in leaves and oil in seeds. Among kenaf components, the leaves possess the highest protein content, which is important for forage production (Swingle *et al.*, 1978; Phillips *et al.*, 2002; Webber *et al.*, 2002; Sanchez *et al.*, 2010).

In 2012, the world's kenaf production was 3,460,500 tons (FAO, 2012). In Malaysia, kenaf production has shown an increasing trend in recent years and is currently planted as a commercial crop in the country. Its production increased from 1,163 tons in 2005 to 4,185 tons in 2007 (Prime Minister's Department, Malaysia, 2008). This increase has been due to the high interest given by the Government to grow it as a substitute to tobacco (Chan and Ismail, 2009).

In Malaysia, very little is known on kenaf varieties used as a forage crop. The variety used as forage is V36, which is recommended by the Tobacco and Kenaf National Board Malaysia. However, no variety has been fully bred for use as forage in ruminant production. Varietal identification was largely based on morphological and agronomic features. However, genetic information and breeding efforts for improving genotypic characteristics of kenaf are limited. To carry out breeding work, it is necessary to understand the genetic control of forage yield and its quality traits that would help breeders to improve the varieties with good forage traits.

In recent years, plant breeders are interested in producing new varieties using new genetic procedures such as DNA molecular markers to facilitate plant breeding programs (Cheng *et al.*, 2002). Microsatellite markers or simple sequence repeat (SSR) markers are widely used in many crops including rice, maize and wheat for studies of genetic diversity, genetic mapping, genome fingerprinting and marker-assisted selection. Presently, genetic information on kenaf using SSR markers is limited. Foroughi (2012) conducted a study on genetic diversity of kenaf. There

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was however, no report on the use of SSR markers for kenaf forage yield and quality.

Hence, the objectives of the present study were to:

1. Evaluate various kenaf varieties for forage yield and quality,
2. Estimate heritability, heterosis and genetic parameters involved in the control of forage yield and quality in kenaf populations using generation means analysis, and
3. Identify the genetic factors associated with kenaf forage yield and quality using SSR markers

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