UNIVERSITI PUTRA MALAYSIA

GENETIC CONTROL OF FIBER YIELD AND QUALITY, AND DNA MARKER DEVELOPMENT IN KENAF (*Hibiscus cannabinus* L.)

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2013

GENETIC CONTROL OF FIBER YIELD AND QUALITY, AND DNA MARKER DEVELOPMENT IN KENAF (*Hibiscus cannabinus* L.)

By

RAHMATOLLAH BEHMARAM

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

April 2013

Dedicated with love to

My dear wife, *Ayse*,
My little angels *Hasan* and *Ayana*,
My late father, *Halim*, and
My kind mother, *Aysheh*

for their endless love, support and sacrifices.

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

GENETIC CONTROL OF FIBER YIELD AND QUALITY, AND DNA MARKER DEVELOPMENT IN KENAF (*Hibiscus cannabinus* L.)

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April 2013

Chairman: Professor Ghizan Saleh, PhD

Faculty: Agriculture

The study was conducted to investigate the genetics of kenaf fiber yield and quality, and identify molecular markers associated with yield and fiber quality traits to facilitate marker-assisted breeding. This study was conducted in three stages. In stage one, two populations which served as base generations were generated, and genetic control and heritability of fiber yield and quality were studied using Analysis of Generation Means procedure. In stage two, kenaf genomic DNA extraction protocol was optimized and new Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) markers were developed. Finally, in stage three, genotyping of individual plants of the F_2 generation and marker-trait association analysis were conducted. Two sets of generations derived

from 1X51 x Ghana07 and Gregg x Ghana07 crosses, i.e. the P_1, P_2, F_1, F_2, $BC_{1.}P_1$ and $BC_{1.}P_2$, were evaluated in a randomized complete block design with three replications. Analysis of generation means using weighted least squares procedure was performed on stalk dry weight, bast percentage, days to flowering, plant height, basal stalk diameter and middle stalk diameter. Analysis of generation means revealed that bast percentage was mainly controlled by dominance effects whereas stalk dry weight was mainly controlled by additive effects. Estimates of heterosis based on mid-parental values were generally high and ranged from 10 to 55% for stalk dry weight and bast percentage. Estimates of inbreeding depression, calculated from F_1 and F_2 generation means, were 55% for stalk dry weight in Population 1 (i.e. derived from 1X51 x Ghana07 cross) and 1.43% in Population 2 (i.e. derived from the Gregg x Ghana07 cross). Estimates of inbreeding depression for bast percentage were 5% in Population 1 and 15% in Population 2. DNA extraction protocol was optimized by adjustment of the cetyltrimethylammonium bromide (CTAB), polyvinylpyrrolidone (PVP) and 2-mercaptoethanol (2-ME) concentrations. The extraction buffer containing 14.3 mM 2-ME and 0.5% CTAB (w/v) produced high DNA quantity and quality. Among 72 SSR primer pairs, 42 primers could amplify fragments from kenaf genomic DNA. Alignment of sequences for templates produced by 15 primers from 1X51 and Ghana07 cultivars revealed Single Nucleotide Polymorphism (SNP). Linkage map was constructed using the marker segregation data obtained from HRM

analysis. Ten markers were located in three linkage groups, while five markers were not assigned to any group. The linkage map contained three linkage groups with 419.3 cM genome coverage. For the first time, a QTL is detected and report in kenaf for days to flowering between SSR-kenaf-24 and AB242153 loci. The QTL that was associated with days to flowering could be useful in future marker assisted breeding studies. The results of this study indicated that, the portion of phenotypic variations, which is controlled by additive gene effects, was generally high for stalk dry weight. Thus, selection in the segregating generations would lead to significant improvement of fiber yield. Furthermore, new procedures of DNA extraction and HRM-SNP F_2 genotyping developed in this study can expedite future kenaf breeding.

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

KAWALAN GENETIK UNTUK HASIL DAN KUALITI GENTIAN, SERTA PEMBANGUNAN PENANDA DNA UNTUK KENAF (*Hibiscus cannabinus* L.)

Oleh

RAHMATOLLAH BEHMARAM

April 2013

Pengerusi: Professor Ghizan Saleh, PhD

Fakulti: Pertanian

Kajian ini dijalankan untuk menyiasat pengawalan genetik untuk hasil dan kualiti gentian kenaf, dan mengenalpasti penanda molekul yang berkait dengan hasil dan ciri kualiti gentian untuk membolehkan pembiakbakaan berbantu penanda dilakukan. Kajian ini telah dijalankan dalam tiga peringkat. Dalam peringkat pertama, dua populasi yang mengandungi generasi asas telah dihasilkan, dan pengawalan genetik serta kebolehwarisan untuk hasil dan kualiti gentian telah dikaji menggunakan prosedur Analisis Min Generasi. Dalam peringkat kedua, protokol untuk pengekstrakan DNA genomik dari kenaf telah dioptimumkan dan penanda baru ‘‘Simple Sequence Repeat’’ (SSR) dan ‘‘Single Nucloetide Polymorphism’’ (SNP) telah dibangunkan. Akhir sekali, dalam peringkat ketiga, penggenotipan setiap pokok dalam generasi F_2 dan analisis

perkaitan antara penanda dan ciri telah dijalankan. Dua set generasi asas yang terhasil dari kacukan antara 1X51 x Ghana07 dan Gregg x Ghana07, iaitu P_1, P_2, F_1, F_2, $BC_{1.}P_1$ dan $BC_{1.}P_2$, telah dinilai menggunakan reka bentuk blok berawak lengkap dengan tiga replikasi. Analisis min generasi menggunakan prosedur 'weighted least squares' telah dilakukan untuk ciri-ciri berat kering batang, peratusan kulit, hari untuk berbunga, tinggi pokok, diameter pangkal pokok dan diameter tengah pokok. Analisis ini telah menunjukkan bahawa peratusan kulit sebahagian besarnya dikawal oleh kesan dominan, manakala berat kering batang sebahagian besarnya dikawal oleh kesan penambah. Anggaran heterosis berdasarkan nilai pertengahan induk pada amnya didapati tinggi dan dalam julat 10 hingga 55% untuk ciri berat kering batang dan peratusan kulit. Anggaran untuk kemelesetan penginbredan, yang dikira berdasarkan min generasi F_1 dan F_2, ialah 55% untuk berat kering batang dalam Populasi 1 (iaitu dihasilkan dari kacukan 1X51 x Ghana07) dan 1.43% untuk Populasi 2 (iaitu dihasilkan dari kacukan Gregg x Ghana07). Anggaran kemelesetan penginbredan untuk peratusan kulit pula ialah 5% dalam Populasi 1 dan 15% dalam Populasi 2. Protokol pengektrakan DNA telah dioptimumkan dengan mengubah kepekatan cetyltrimethylammonium bromide (CTAB), polyvinylpyrrolidone (PVP) dan 2-mercaptoethanol (2-ME). Penampan pengekstrakan yang mengandungi 14.3 mM 2-ME dan 0.5% CTAB (w/v) menghasilkan DNA dengan kuantiti dan kualiti yang tinggi. Di antara 72 pasang SSR primer, 42 primer didapati mampu menggandakan fragmen

DNA genomik dari kenaf. Penjajaran jujukan templat yang dihasilkan oleh 15 primer daripada varieti 1X51 dan Ghana07 menunjukkan terdapatnya Polimorfisma Satu Nukleotida (SNP). Peta rangkaian telah dibina menggunakan data segregasi penanda yang diperolehi daripada analisis HRM. Sepuluh penanda terletak di dalam tiga kumpulan rangkaian, manakala lima penanda tidak terletak di dalam mana-mana kumpulan. Peta rangkaian tersebut mengandungi tiga kumpulan rangkaian dengan liputan genom 419.3 cM. Untuk pertama kalinya, satu QTL berjaya dikesan dan dilaporkan dalam kenaf untuk ciri hari untuk berbunga di antara lokus SSR-kenaf-24 dan AB242153. QTL yang dikaitkan dengan ciri hari untuk berbunga tersebut mungkin berguna dalam kajian pembiakbakaan berbantu penanda pada masa hadapan. Keputusan kajian ini menunjukkan bahawa, bahagian variasi fenotip, yang dikawal oleh kesan gen penambah, secara amnya tinggi untuk ciri berat kering batang. Oleh itu, pemilihan untuk generasi bersegregasi akan membawa kepada peningkatan yang ketara untuk penghasilan gentian. Tambahan itu, prosedur baru untuk pengektrakan DNA dan pengenotipan F_2 HRM-SNP yang dihasilkan dalam kajian ini boleh mempercepatkan kerja-kerja pembiakbakaan kenaf pada masa akan datang.

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I certify that an Examination Committee has met on 26 April 2013 to conduct the final examination of Rahmatollah Behmaram on his PhD thesis entitled “Genetic Control of Fiber Yield and Quality, and DNA Marker Development in Kenaf (Hibiscus cannabinus L.)” 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. The Committee recommends that the student be awarded the PhD.

Members of the Thesis Examination Committee were as follows:

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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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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.

RAHMATOLLAH BEHMARAM

Date: 26 April 2013

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