GENETIC DIVERSITY AND DEVELOPMENT OF HIGH FIBER YIELD KENAF (*Hibiscus cannabinus* L.) MUTANT HYBRIDS THROUGH DIALLEL CROSSES

By

MD. AL-MAMUN

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

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DEDICATIONS

This thesis is dedicated to my father and mother, who infused in me the spirit of studying and whose blessing inspired me to achieve higher life goals. I also dedicated it to the people of my hometown for inspiring me to continue my education.

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

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Chairman : Professor Mohd Rafii bin Yusop, PhD
Institute : Tropical Agriculture and Food Security

Kenaf (*Hibiscus cannabinus* L.) is an economically important fiber crop globally for multipurpose industrial uses such as paper making, car interior components and building boards. However, commercial cultivation of this fiber crop in Malaysia has limitations due to lack of superior varieties with high fiber yield. This study was conducted to assess the genetic diversity among kenaf mutants based on agro-morphological traits and molecular markers diversity, analyze the combining ability of the selected parents and hybrids, and estimate heterosis and the nature of gene actions. To achieve these objectives, field trials and molecular diversity works were conducted at Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, between 2019 and 2021. Data were collected on the eight qualitative and 15 quantitative traits of the 31 kenaf genotypes. The analysis of variance over two evaluation seasons showed significant differences among genotypes for all traits except for stem top diameter. According to principal component analysis (PCA), the first five PCA accounted for 85.16% variation between genotypes based on a correlation matrix of all the quantitative traits. The mutant lines derived from V36 parental variety were grouped into six clusters, indicating a wide range of genetic variability. Path coefficient analysis revealed the maximum contribution of fresh stem weight without leaves and pod to fiber yield, followed by plant height. For molecular characterization, 10 out of the 72 expressed sequence tags - simple sequence repeat (EST-SSR) primers produced clear polymorphic bands with a mean value of 5.2 alleles per primer. These EST-SSR primers were found to be extremely informative, suggesting that among the 31 genotypes were genetically diverse. The polymorphism information content (PIC) value ranged from 0.531 to 0.737, with a mean of 0.610, indicating that the genotypes are genetically diverse. Shannon index estimation varied from 0.982 to 1.515 among 31 genotypes, whereas 0.123 to 0.405 was recorded within the early, intermediate and mid-late flowering groups. Genetic differentiation ranged from 0.67 to 1.0 alongside the average gene flow (Nm) of 0.024. The analysis of molecular variance (AMOVA) revealed 76% variation within three flowering groups., while among the groups recorded was 24%. Based on the unweighted pair group method with arithmetic means (UPGMA) dendrogram the evaluated kenaf mutants were clustered into five major groups based on EST-microsatellites data. Combining phenotypic and molecular data in the selection of parents for hybridization activities would yield a more accurate summary and the cluster analysis identified eight mutant lines (G5, G9, G13, G21, G22, G24, G30 and G31) and one inbred (G28) that were selected for hybrid crossing using a half diallel method. The genotypes were chosen as parental sources from each heterotic group at the genetic and phenotypic levels on genetic distances and several important agronomic traits. Thirty-six hybrids were successfully produced from nine morphologically distinct kenaf genotypes using half diallel mating design. Field trials were carried out in two environments to evaluate the yield performance of 36 F_1 hybrid crosses and nine parental lines using seven qualitative and 15 quantitative attributes. From the combined data of the two environments, the combining ability analysis revealed significant general combining ability (GCA) for all traits except stem top diameter, and significant specific combining ability (SCA) for all traits except plant height and stem top diameter. The result indicated that additive and non-additive gene actions were involved in the genetic control of the traits. The magnitude of GCA variance was considerably higher than that of SCA variance except for stem top diameter, node number, and fresh stem weight with leaves and pod, showing that additive gene action predominates for these traits. The parental lines P_1 (G5: ML5), P_3 (G13: ML36-10) and P_4 (G21: ML36-24) had outstanding general combiners for fiber yield and yield-related traits. Most traits showed over-dominant gene action, except for plant height, stem middle diameter, stem top diameter, days to first flowering, dry stick weight, dry fiber weight and 1000 seed weight, which showed a partial dominance. Considering specific combining ability and heterosis values, the crosses P_1 (G5) × P_4 (G21), P_1 (G5) × P_9 (G31), P_2 (G9) × P_3 (G13), P_2 (G9) × P_5 (G22), P_4 (G21) × P_6 (G24), P_4 (G21) × P_7 (G28), P_4 (G21) × P_9 (G31), P_5 (G22) × P_8 (G30), and P_7 (G28) × P_9 (G31) were high heterotic response for fiber yield, stick yield, number of pods per plant and smaller seed size. The nine hybrids are recommended for further evaluation at multi-location trials to select several superior hybrids with high fiber yield for commercial cultivation in Malaysia.

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

KEPELBAGAIAN GENETIK DAN PEMBANGUNAN HIBRID MUTAN KENAF (*Hibiscus cannabinus* L.) BERHASIL FIBER TINGGI MELALUI KACUKAN DIALLEL

Oleh

MD AL-MAMUN

Ogos 2022

Pengerusi : Profesor Mohd Rafii bin Yusop, PhD
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Kenaf (*Hibiscus cannabinus* L.) ialah tanaman fiber yang penting dari segi ekonomi di peringkat global untuk kegunaan industri pelbagai guna seperti pembuatan kertas, komponen dalaman kereta dan papan bangunan. Walau bagaimanapun, penanaman secara komersial tanaman fiber ini di Malaysia mempunyai kekangan kerana kekurangan varieti unggul dengan hasil fiber yang tinggi. Kajian ini telah dijalankan untuk menilai kepelbagaian genetik antara mutan kenaf berdasarkan ciri-ciri agro-morfologi dan kepelbagaian penanda molekular, menganalisis keupayaan gabungan induk dan hibrid terpilih, serta menganggarkan heterosis dan kesan tindakan gen. Untuk mencapai objektif ini, kajian di lapangan dan percubaan kepelbagaian molekul telah dijalankan di Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia, antara 2019 dan 2021. Data telah dicerap untuk lapan ciri kualitatif dan 15 ciri kuantitatif ke atas 31 genotip kenaf. Analisis varians sepanjang dua musim penilaian menunjukkan perbezaan yang signifikan antara genotip untuk semua ciri kecuali diameter atas batang. Menurut analisis komponen utama (PCA), lima PCA pertama menyumbang 85.16% variasi antara genotip berdasarkan matriks korelasi semua ciri-ciri kuantitatif. Titisan mutan yang terhasil dari varieti induk V36 telah diklusterkan kepada enam kelompok, menunjukkan kepelbagaian genetik yang luas antara mutan tersebut. Analisis pekali laluan menunjukkan sumbangan maksimum berat batang segar tanpa daun dan buah terhadap hasil fiber, dan diikuti dengan tinggi pokok. Untuk pencirian molekul, 10 daripada 72 primer penanda jujukan terekspres – jujukan ringkas berulang (EST-SSR) menghasilkan jalur polimorfik yang jelas dengan nilai min 5.2 alel bagi setiap penanda. Penanda EST-SSR ini didapati sangat informatif, yang menunjukkan bahawa di kalangan 31 genotip mempunyai kepelbagaian genetik. Nilai kandungan maklumat polimorfisma (PIC) adalah antara 0.531 hingga 0.737, dengan nilai min 0.610 yang menunjukkan genotip tersebut mempunyai kepelbagaian genetik. Anggaran indeks Shannon berbeza dari 0.982 hingga 1.515 dalam kalangan 31 genotip, manakala 0.123 hingga 0.405 direkodkan dalam kumpulan berbunga awal, pertengahan dan pertengahan akhir. Pembezaan genetik dengan julat antara 0.67 hingga 1.0, beserta 0.024 aliran gen purata (Nm) telah direkodkan. Analisis varians molekul (AMOVA) memberikan 76% variasi di dalam tiga kumpulan berbunga.,

manakala antara kumpulan pula yang direkodkan ialah 24%. Berdasarkan kaedah dendrogram kumpulan pasangan tak berwajaran dengan cara aritmetik (UPGMA), mutan kenaf yang dinilai telah diklusterkan kepada lima kumpulan utama berdasarkan data EST-microsatellites. Gabungan data fenotip dan molekul dalam pemilihan induk untuk aktiviti hibridisasi akan menghasilkan rumusan yang lebih tepat dan analisis kluster telah mengenalpasti lapan titisan mutan (G5, G9, G13, G21, G22, G24, G30 and G31) dan satu inbred (G28) yang telah dipilih untuk hibrid mengunakan kaedah separa dialel. Genotip tersebut telah dipilih sebagai sumber induk daripada setiap kumpulan heterotik di peringkat genetik dan fenotip bagi jarak genetik dan beberapa ciri agronomik yang penting. Tiga puluh enam kacukan telah berjaya dihasilkan daripada sembilan genotip kenaf yang berbeza secara morfologi menggunakan reka bentuk mengawan separuh dialel. Lain kajian lapangan telah dijalankan di dua persekitaran bagi menilai hasil prestasi kacukan 36 hibrid F_1 dan sembilan titisan induk menggunakan tujuh ciri kualitatif dan 15 ciri kuantitatif. Berdasarkan kepada gabungan data di dua persekitaran, analisis keupayaan bergabung mendapati keupayaan bergabung am (GCA) adalah signifikan untuk semua ciri kecuali diameter batang atas, manakala keupayaan bergabung khusus (SCA) pula signifikan untuk semua ciri kecuali ketinggian tumbuhan dan diameter batang atas. Keputusan ini menunjukkan bahawa tindakan gen aditif dan bukan-aditif terlibat dalam kawalan genetik bagi ciri-ciri tersebut. Magnitud varians GCA adalah jauh lebih tinggi daripada varians SCA kecuali diameter batang atas, bilangan nod, dan berat batang segar dengan daun dan buah, menunjukkan bahawa tindakan gen aditif yang mendominasi ciri-ciri ini. Induk P_1 (G5: ML5), P_3 (G13: ML36-10) and P_4 (G21: ML36-24) mempunyai keupayaan bergabung am yang tinggi untuk hasil fiber dan ciri komponen hasil yang lain. Kebanyakan ciri menunjukkan tindakan gen dominan lampau, kecuali ketinggian pokok, diameter batang tengah, diameter batang atas, hari berbunga pertama, berat batang kering, berat fiber kering dan berat 1000 biji, yang menunjukkan dominan separa. Mengambil kira keupayaan bergabung khusus dan nilai heterosis, hibrid P_1 (G5) × P_4 (G21), P_1 (G5) × P_9 (G31), P_2 (G9) × P_3 (G13), P_2 (G9) × P_5 (G22), P_4 (G21) × P_6 (G24), P_4 (G21) × P_7 (G28), P_4 (G21) × P_9 (G31), P_5 (G22) × P_8 (G30) dan P_7 (G28) × P_9 (G31) didapati memberikan nilai heterotik yang tinggi terhadap hasil fiber, hasil kayu, bilangan buah setiap tumbuhan dan saiz benih yang lebih kecil. Sembilan hibrid ini disyorkan untuk penilaian selanjutnya di percubaan berbilang lokasi bagi memilih beberapa hibrid unggul dengan hasil fiber yang tinggi untuk penanaman komersial di Malaysia.

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

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This is to confirm that:
- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in Rule 41 in Rules 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
Avg. He	Average heterozygosity
Bfb	Black with few brownish
BJRI	Bangladesh Jute Research Institute
Bp	Base pairs
BP	Mean value of better parent
CC	Completely cream with white stigma
CD	Core diameter
Cpc	Cream with purple Centre
CTAB	Cetyltrimethylammonium Bromide
CV	Coefficient of variation
D50%F	Days to 50% flowering
DAS	Days after sowing
DF	Degrees of freedom
DFW	Dry fiber weight
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxy nucleotide triphosphate
DSW	Dry stick weight
DTFF	Days to first flowering
EDTA	Ethylenediaminetetraacetic acid
EMS	Error Means Square

EST	Expressed Sequence Tag
FAO	Food and Agriculture Organization of the United Nations
Fst	Genetic differentiation
FW1	Fresh stem weight with leaves and pod
FW2	Fresh stem weight without leaves and pod
G × E	Genotype × environmental interaction
GCA	General Combining Ability
Grp	Green with reddish patches
Gy	Gray
H_1	Dominance
H_2	Dominance × dominance
h^2	Overall dominance
h^2_B	Heritability in broad sense
h^2_N	Heritability in narrow sense
HCI	Hydrochloric acid
He	Expected heterozygosity
Ho	Observed heterozygosity
IAEA	International Atomic Energy Agency
IJO	International Jute Organization
LD_{50}	50% lethal dose
LSD	Least Significant Difference
M_1	First generation after mutagenic treatment
M_2	Second generation after mutagenic treatment
M_3	Third generation after mutagenic treatment
M_4	Fourth generation after mutagenic treatment

M_5	Fifth generation after mutagenic treatment
M_6	Sixth generation after mutagenic treatment
M_7	Seventh generation after mutagenic treatment
mm	Millimeter (s)
mM	MilliMolar
MNA	Malaysian Nuclear Agency
MP	Mean value of two parental involves in F_1
MVD	Mutant Variety Database
Na	Average observed number of alleles
Ne	Average effective number of alleles
NF	Number of pods per plant
ng	Nano gram
Nm	Estimate of gene flow among populations
nm	Nano meter
NN	Number of nodes
NS	Non-significant
NS	Number of seeds per pod
OA Mean	Overall mean
PC	Principal component
PCA	Principal component analysis
PCO	Principal co-ordinate analysis
PCR	Polymerase Chain Reaction
PH	Plant height
PIC	Polymorphism Information Content
RaGb	Reddish above greenish below

RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
S.O.V	Source of variation
SAS	Statistical Analysis System
SBD	Stem base diameter
SDS	Sodium dodecyl sulphate
SE	Standard error
SI	Shannon's information index
SMD	Stem middle diameter
SSR	Simple sequence repeat
STD	Stem top diameter
SW	1000 seeds weight
Taq	Thermus aquaticus
TBE	Tris borate EDTA
TE	Tris/EDTA
TR	Trace reddish
Tris	Tris (hydroxymethyl) amino methane
uHe	Unbiased expected heterozygosity
UPGMA	Unweighted pairs group method of arithmetic mean
UPM	Universiti Putra Malaysia
UrLg	Upper surface light reddish but lower surface green
UV	Ultraviolet
µl	Microliter
µM	Micromolar

CHAPTER 1

INTRODUCTION

1.1 General introduction

Kenaf (*Hibiscus cannabinus* L.) is an industrial crop belonging to the Malvaceae family and the genus *Hibiscus*. There are over 200 species in this family including hibiscus (*Hibiscus hibiscum* L.), hollyhock (*Althaea rosea*), cotton (*Gossypium hirsutum* L.), and okra (*Hibiscus esculentus*) that are commercially grown in several countries, including Malaysia. Kenaf is a dicot plant with two distinct fiber types: short core fibers, which make up 70 to 75% of the dry weight of the stalk, and long bark fibers, which make up the remaining 25 to 30% of the fiber component (Wong et al., 2008; Lips et al., 2009). Kenaf is a multipurpose crop that produces biomass for energy and natural fiber for industrial uses, owing to its height growth and fiber content (Dauda et al., 2013). The protein content of kenaf leaves suggests that it could be used as a high-protein base feed for livestock (Hollowell et al., 1997). Because of increased concerns about global warming and the rising cost of petroleum-based products, kenaf has been praised as the fiber crop of the twenty-first century, with a short breeding season of four months (Saba et al., 2015a).

Kenaf is one of the most essential crops cultivated for its smooth fiber production. It has exceptional air permeability, antibacterial properties, and excellent biological qualities such as salinity tolerance, drought resistance, wide adaptability, and high yield. The key factor for effective use of this natural fiber material is to ensure the sustenance of high-quality raw materials to meet the growing global demands. Hence, understanding the genetic basis for kenaf planting materials is a prerequisite for a successful breeding program.

Genetic variability is a dynamic property of germplasm, and it can be estimated using molecular, biochemical, and morphological assessment (Oladosu et al., 2015). Among the three methods, morphological evaluation is the most cost-effective and readily quantifiable, making it the best alternative for crop improvement programs. In comparison to conventional breeding, mutation induction is the best way to develop new varieties with desirable traits within a short period (Oladosu et al., 2016). Correlation coefficient studies between characters are instrumental in determining effective breeding procedures (Tulu, 2014). Similarly, selection and recommendation for commercial cultivation are only proper after comprehensive information and research of available germplasm.

Simple sequence repeats (SSRs) or Microsatellites have shown clear superiority over other molecular markers due to high reproducibility and abundance, easy scoring, co-dominant nature, and extensive coverage. SSRs are classified into genomic SSRs (gSSRs) and expressed sequence tag SSRs (EST-SSRs) based on their source. Genomic SSRs are usually related to non-coding parts, while EST-SSRs are derived from the genome expressed parts (Li et al., 2004). Germplasm evaluation using SSRs derived from ESTs may improve the role of genetic markers by analyzing variations in transcribed and known function genes (Datta et al., 2021). Therefore, EST-SSRs are among the important genetic markers for marker-assisted breeding, high-density genetic mapping, and analysis of genetic diversity (Li et al., 2016). In addition, the transcribed region of the genome contains EST-SSRs, which may be relatively well conserved and represent a better relationship between species or varieties.

Compared to alternative mating designs, the diallel analysis is an effective method for screening parents for hybrid production. General combining ability (GCA) refers to a parent's average performance in a series of crossings. Parents with a high GCA effect have additive gene action, but they do not always have a favorable SCA in their combination (Santha et al., 2017). Meanwhile, determining the sort of gene action that affects the phenotypes of interest using SCA estimation is useful in genetic research. A high SCA identifies non-additive gene action (Virmani, 2003). SCA and GCA data aid in selecting hybrids and parents for successful breeding (Patel et al., 2013). Therefore, strong hybrids are produced by parents who have good general combining ability (Shattuck et al., 1993).

1.2 Problem Statement

As part of the ASEAN Free Trade Area (AFTA), the Malaysian government encouraged kenaf planting to replace tobacco to reduce tobacco import duties by 2010 (Kamal, 2014). Despite its wide distribution in tropical countries, kenaf productivity in Malaysia is low due to a lack of high-yielding varieties with fiber production of around 5 to 10 tons per hectare and research into developing new kenaf varieties is still lacking (Sani et al., 2017). Developing new kenaf varieties that produce high biomass is vital for effective kenaf production (Al-Mamun and Saha, 2017). Thus, breeding kenaf in Malaysia is essential for producing high fiber content and adaptable growth in the local climates (Sani et al., 2017).

In some countries, such as China, Russia, and Thailand, kenaf hybrids are cultivated commercially, contributing to increasing production (Liu, 2005). In Malaysia, hybrid kenaf technology is considered novel and more research is required to assess genetics, agronomy, and crop management. A hybrid kenaf breeding program is required to develop a high-yielding, stable performance hybrid kenaf for the Malaysian environment. The availability of high-quality jute and kenaf seed would help ensure fiber quality to a large extent (Al-Mamun et al., 2017). As a result, developing locally adapted hybrid kenaf seeds is a viable alternative for enhancing national kenaf yield and increasing kenaf producer income.

1.3 Research objectives

1.3.1 Main Objective

To create stable kenaf hybrids with high fiber yield for commercial cultivation.

1.3.2 Specific Objectives

i. To determine the genetic variability, heritability, and paths of influence among various yield components of selected kenaf mutants and inbred lines for high fiber yield.

ii. To analyze the phylogenetic relationship and genetic divergence among kenaf mutants and inbred lines using molecular and agronomic characterizations to select potential parents for heterotic hybridization.

iii. To estimate the nature of gene action and the magnitude of heterosis, assess the combining ability of selected parents and their hybrids.

iv. To select stable and high yielding kenaf hybrids for dual-purpose (fiber and seeds) commercial cultivars in Malaysia's hot climate.

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