UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY AND SELECTION OF HIGH-YIELDING GENOTYPES FROM THE AMAZONIAN HEVEA GERMPLASM FOR UTILISATION IN RUBBER BREEDING PROGRAMME

MOHD ADI FAIZ BIN AHMAD FAUZI

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By

MOHD ADI FAIZ BIN AHMAD FAUZI

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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DEDICATION

This thesis is dedicated to my parent, Ahmad Fauzi bin Mohamed and Faridah binti Taib. Without their love, endless support, and encouragement I would never have been able to complete this journey.

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 Yusop, PhD
Institute : Tropical Agriculture and Food Security

Systematic initiatives in evaluation of *Hevea* germplasm are imperative in the development of improved cultivars with desirable characters. The main objective of the present study was to evaluate genetic diversity of the Amazonian *Hevea* germplasm in efforts to establish a breeding population consisting of selected genotypes with desirable traits for their utilisation in future rubber breeding programme. A total of 4,545 wild *Hevea brasiliensis* genotypes from eight Amazonas and Para populations, constituted the base materials for the present study. Evaluation on genetic diversity revealed significant variations in latex yield and vegetative traits among the genotypes. Majority of the wild genotypes produced considerably low amount of latex yield. Higher phenotypic coefficient of variations was observed for all traits compared to genotypic coefficient of variations, suggesting major effects of environmental factors on the expression of vegetative and yield traits. Latex yield recorded the highest magnitude of heritability and genetic advance, indicating that selection of genotypes based on this trait would give good selection response. The highest genetic distance was recorded between G95_MA and G95_ADN populations, while the lowest was between G95_BC and G95_ADN populations. Cluster analysis based on quantitative traits divided the eight populations into three clusters. The first two components in the principal component analysis accounted for approximately 86.90% of total variations. A total of 505 potential genotypes with desired characteristic were initially selected using multiple traits selection index and the relationship between latex yield, and morphological and bark structural traits were further evaluated. Latex yield showed significant and positive phenotypic correlations with plant girth, total number of latex vessel rings, total bark thickness and total leaf area. Plant girth recorded the highest direct positive effects on latex yield advocating its use as an indirect selection criterion towards increasing yield. Based on the principal component scores, Shannon-Weaver diversity index and Gower metric coefficient, a total of 128 genotypes were included in the final core collection. Furthermore, 78 genotypes were evaluated for resistance to two virulent isolates of *Corynespora cassiicola* (isolates CSB 16 and CLN 16) under both laboratory and

greenhouse conditions. Significant differences were observed. Three genotypes had high level of resistance to both isolates in both conditions which could serve as good source of materials in breeding programmes. Results of the study present the genetic potential of the Amazonian *Hevea* germplasm for utilisation in future development of superior latex timber clones.

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

KEPELBAGAIAN GENETIK DAN PEMILIHAN GENOTIP HASIL TINGGI DARI GERMPLASMA *HEVEA* AMAZONIA BAGI KEGUNAAN DALAM PROGRAM PEMBIAKBAKAAN GETAH

Oleh

MOHD ADI FAIZ BIN AHMAD FAUZI

Januari 2021

Pengerusi : Profesor Mohd Rafii Yusop, PhD
Fakulti : Pertanian Tropika dan Sekuriti Makanan

Usaha yang sistematik dalam penilaian germplasma *Hevea* adalah penting bagi membangunkan kultivar yang ditambah baik dengan kecirian yang diingini. Objektif utama kajian adalah untuk menilai kepelbagaian genetik germplasma *Hevea* Amazonia dalam usaha membentuk satu populasi pembiakbakaan yang mengandungi genotip terpilih dengan kecirian yang diingini bagi kegunaan dalam program pembiakbakaan getah di masa depan. Sejumlah 4,545 genotip liar *Hevea brasiliensis* daripada populasi Amazonas dan Para telah dijadikan bahan asas kajian. Penilaian terhadap kepelbagaian genetik menunjukkan variasi ketara untuk ciri hasil lateks dan vegetatif di kalangan genotip. Kebanyakan genotip liar memberi hasil lateks yang rendah. Pekali variasi fenotip yang tinggi telah direkodkan bagi kesemua kecirian berbanding pekali variasi genotip, menunjukkan kesan utama faktor persekitaran ke atas ekspresi kecirian vegetatif dan hasil lateks. Hasil lateks merekodkan nilai heritabiliti dan kemajuan genetik yang tertinggi memberi makna bahawa pemilihan genotip berdasarkan kecirian ini akan memberikan tindakbalas yang baik. Jarak genetik yang tertinggi telah direkodkan antara populasi G95_MA dan G95_ADN, sementara jarak terendah adalah antara populasi G95_BC dan G95_ADN. Analisis kluster berdasarkan kecirian kuantitatif membahagikan lapan populasi kepada tiga kluster. Dua komponen pertama di dalam analisis komponen utama menyumbang kepada anggaran 86.90% daripada jumlah variasi. Sejumlah 505 genotip berpotensi tinggi dengan kecirian diingini telah dipilih pada awalnya menggunakan indeks pemilihan pelbagai kecirian dan hubungan antara hasil lateks dan sifat morfologi serta struktur kulit seterusnya telah dinilai. Hasil lateks menunjukkan korelasi fenotip yang ketara dan positif bagi lilitan tanaman, jumlah bilangan gelung saluran lateks, jumlah ketebalan kulit dan jumlah keluasan daun. Lilitan tanaman merekodkan kesan langsung yang positif ke atas hasil lateks mengukuhkan kegunaannya sebagai kriteria pemilihan tidak langsung ke arah peningkatan hasil. Berdasarkan skor komponen utama, indeks kepelbagaian Shannon-Weaver dan pekali metrik Gower, sejumlah 128 genotip telah dimasukkan sebagai koleksi teras akhir. Selanjutnya, 78 genotip telah dinilai terhadap ketahanan kepada dua isolat virulen dari

dua strain *Corynespora cassiicola* (isolat CSB 16 and CLN 16) di bawah persekitaran makmal dan rumah hijau. Perbezaan yang ketara telah direkodkan. Tiga genotip mempamerkan tahap rintangan yang tinggi terhadap kedua isolat virulen tersebut dan boleh dijadikan sebagai bahan sumber bagi program pembiakbakaan yang baik. Hasil kajian telah menunjukkan potensi genetik germplasma *Hevea* Amazonia bagi kegunaan dalam membangunkan klon lateks balak yang unggul di masa hadapan.

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

Mohd Rafii Yusop, PhD
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Chairman)

Zulkefly Sulaiman, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Nusaibah Syd Ali, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name of Chairman of Supervisory Committee: Professor Dr. Mohd Rafii Yusop

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Zulkefly Sulaiman

Signature: _____
Name of Member of Supervisory Committee: Dr. Nusaibah Syd Ali

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LIST OF ABBREVIATIONS

A	Amazonian
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ANRPC	Association of Natural Rubber Producing Countries
AUDPC	Area under disease-progress curve
AV	AVROS
AVROS	Algenene Vereniging Rubber Planters
BPP	Balai Penelitian Perkebunan
CLD	*Corynespora* leaf disease
CRD	Completely randomized design
D^2	Mahalanobis distance
DAI	Days after inoculation
DSI	Disease severity index
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
FAA	formalin-acetic-70% alcohols
FRIM	Forest Research Institute of Malaysia
FX	Ford crosses Eastern
GA	Expected genetic advance
GAM	Generation-wise Assortative Mating
GCV	Genotypic coefficient of variation
Gl	Glenshiel
GT	Gondang Tapen
g/t/t	gram/tree/tapping

h_B^2	Heritability in broad sense
HPST	Hand Pollinated Seedlings Trial
IAN	Institute Agronomico do Norte
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IRCI	Institute des Recherches sur le Caoutchouc en Indochine
IRRDB	International Rubber Research and Development Board
IRSG	International Rubber Study Group
JPSM	Forestry Department Peninsular Malaysia
Kg/ha/year	Kilogram/hectare/year
LCB	Lands Caoutchouc Bedrijven
LSCT	Large Scale Clone Trial
LSD	Least significant difference
MRB	Malaysian Rubber Board
MTC	Malaysian Timber Council
PB	Prang Besar
PBFP	Prang Besar Further Proof
PBIG/GG	Prang Besar Isolation Garden/ Gough Garden
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PDI	Percent disease intensity
Pil	Pilmoor
PR	Proefstation voor Rubber
PSA	Potato sucrose agar
RCBD	Randomized complete block design

REML	Restricted maximum likelihood
RRIC	Rubber Research Institute of Ceylon
RRII	Rubber Research Institute of India
RRIM	Rubber Research Institute of Malaysia
RRISL	Rubber Research Institute of Sri Lanka
SALB	South American Leaf Blight
SI	Selection index
SSCT	Small Scale Clone Trial
S/2 d3	Half spiral cut downward, tapped once every three days
S/2 d3 6d/7	Half spiral cut downward, tapped once every three days, six days of tapping followed by one day rest
Tjir	Tjirandji
TPD	Tapping panel dryness
UPGMA	Unweighted paired group method with arithmetic mean
USDA	United States Department of Agriculture
W	Wickham
ΔG	Predicted genetic gain

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Rubber tree, *Hevea brasiliensis* (Muell. Arg.), is one of the most important plantation crops in the world producing an undeniably worldwide beneficial commodity for the past 100 years. Latex, produced from the rubber tree, has been the major contributor to natural rubber industry in the development of various rubber and rubber-based products.

Rubber itself is a hydrocarbon polymer made up of isoprene units. It is a secondary metabolite (1, 4-polyisoprene) primarily originating in the secondary phloem of the tree (Priyadarshan, 2017a). It is known that there is no synthetic substitute which has the elasticity, resilience and high temperature resistance comparable to natural rubber (Davies, 1997).

In 2018, world rubber production and consumption stood at about 13.88 and 13.77 million tonnes, respectively (IRSG, 2019). Approximately 91% of the total global natural rubber production is produced in Asia, followed distantly by Africa at 6.5% and Latin America at 2.5% (IRSG, 2019). At present, the world's leading producers of natural rubber include Thailand, Indonesia, Vietnam, China, India, Cote D'Ivoire and Malaysia (MRB, 2019). In Malaysia, natural rubber has played and will continue to play a dominant role in the country's economy. In the 1930s, she produced half of the world's natural rubber production, but presently, stands as the seventh largest producing country with a production of 603,000 tonnes and a total area of 1.08 million hectares (MRB, 2019). The setback was primarily due to the country's transformation from agricultural-based or resource-based to industrialization economy (Akhbar and Embong, 2012). Nevertheless, the industry has provided the foundation upon which other industries have developed, grown, and prospered.

It has been cited that the present state of the rubber industry in Malaysia owes much to a number of important scientific advances in the fields of breeding and selection of high yielding clones, tapping and stimulation methods, and efficient agro-management practices including fertiliser applications, planting systems and disease control (Abdul Aziz, 2002). The Rubber Research Institute of Malaysia (RRIM), established in 1925, had and continues to play a key role in research and development efforts in the industry. Breeding and selection programmes in RRIM have been instrumental in the development of high yielding clonal materials. The success of rubber breeding and selection programmes is marked by the increase in latex yield from a mere 496 kg/ha/year from unselected plants to more than 2,500 kg/ha/year from recent clones (Dijkman, 1951; Tan, 1987; MRB, 2009).

Although achievement and realisation of yield potential in the field have been remarkable, several problems and concerns associated with the processes of breeding and selection have been recognised as factors or issues hampering future progress in rubber breeding. According to Ho (1979) and Tan (1987), these problems included narrow genetic base, long breeding and selection cycle, choice of suitable parents, selection of multiple desirable characters, disease resistance, genetic by environment interaction, non-synchronization of flowering as well as low fruit sets. Among these, the problem associated with narrow genetic base has become a major concern in rubber breeding.

Realising the importance of broadening the genetic base of *Hevea*, efforts have been taken by rubber breeders to address the issue through introduction of new genetic materials from South America, the native habitat of the genus *Hevea*. In Malaysia, a number of initiatives have been made to broaden the genetic base through introductions of genetic materials undertaken in 1950's, 1966, 1981 and 1995 (Ong et al., 1983; Ong and Tan, 1987; Masahuling and Ramli, 1995). In addition, exchanges of genetic materials have been carried out among rubber growing countries, notably in 1954 and 1974 (RRIM, 1959; Tan, 1987). These efforts have resulted in the improvement of latex yield and other secondary desired traits with the utilisation of parents from diverse genetic resources and geographical origin.

The last expedition to the Amazon basin in 1995, RRIM together with their Brazilian counterparts, Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), succeeded in collecting seeds from various species of *Hevea* genus in the states of Amazonas and Para, Brazil (RRIM, 1997; MRB, 1999). The expedition was with the objective of safeguarding valuable genetic resources and to broaden the genetic base of *Hevea*. The numerous seeds collected were split between Malaysia and Brazil. In Malaysia, a total of 50,231 wild genotypes, mainly being *H. brasiliensis* were successfully cultivated for evaluation and utilisation in the rubber breeding programme (Masahuling and Ramli, 1995; MRB, 1999). To date, the Amazonian *Hevea* germplasm collection is the largest *ex-situ* conservation of *Hevea* genetic materials currently available in Asia.

1.2 Problem Statements

Despite the availability of Amazonian *Hevea* germplasm in the country, the potential of these materials remains relatively unexplored. Since the establishment of the germplasm, none of the genotypes has been incorporated into a breeding programme due to lack of evaluation and characterization of the genotypes. There was no information on latex yield, growth potential, and disease resistance as well as genetic variability of the germplasm. Limited evaluation and characterization have hindered their rapid and effective utilisation. Hence, identification and selection of a collection of genotypes with reduced sample size and enhanced characters are considered as a prerequisite for ultimate utilisation of the germplasm.

1.3 Objectives

The present investigation was carried out to form a breeding population consisting of selected genotypes with desired characteristics for further exploitation and accelerate their utilisation in rubber breeding programme. Specifically, the objectives of the present study were:

i. To quantify phenotypic variability and estimate genetic variances of quantitative traits in the wild *Hevea* germplasm;

ii. To identify potential genotypes with desired characteristics of high latex yield, clear bole volume and vigorous growth;

iii. To ascertain the degree of associations between the different traits with the latex yield and their inter-correlations;

iv. To identify genotypes resistant against virulent *Corynespora cassiicola* isolates;

v. To select superior genotypes for the development of commercial rubber clones.

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BIODATA OF STUDENT

Mohd Adi Faiz bin Ahmad Fauzi was born in Alor Setar, Kedah on 18th September 1986. He had his early education at Mara Junior Science College (MRSM) Jeli, Kelantan. He graduated with a Bachelor's degree in Industrial Statistics (Hons.) in 2009 from Universiti Utara Malaysia. He further earned his Master's degree in Applied Statistics from Universiti Putra Malaysia in 2013. He began his career in 2011 as a Research Officer in Plant Improvement Programme, Biotechnology Division, Forest Research Institute Malaysia (FRIM). While there, he was responsible for conducting research related to breeding of several forest species. He later joined the Malaysian Rubber Board (MRB) in 2013 under the Crop Improvement and Protection Unit, Production Development Division as a Research Officer. He is presently attached to the Genetic Resources and Improvement Unit at MRB. His work in the MRB involves selection, utilization and improvement of *Hevea* genetic resources. He is currently active in conducting research on several *Hevea* species. His work has led to the identification and selection of potential genotypes possessing desired characteristics of high yielding in both latex and timber to be incorporated into breeding programme for future development of superior planting materials. He is also working on identification of genotypes with tolerance to several major diseases in *Hevea*. He has been an active member of the Malaysian Institute of Statistics since 2011 and has recently joined the Malaysian Genetics Association. He has published and presented several scientific papers in journals, international conferences, and workshops. He enjoyed outdoor activities such as jungle trekking and 4 × 4 off-road adventure.

LIST OF PUBLICATIONS

Journals

Adifaiz, A.F., Maiden, N.A., Aizat Shamin, N., Zarawi, A.G. and Rafii, M.Y. (2017). Potential genotypes of the 1995 RRIM *Hevea* germplasm for future rubber breeding and selection programme. *Journal of Rubber Research* 20(4), 242-260.

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Proceedings

Adifaiz, A.F., Maiden, N.A., Aizat Shamin, N., Zarawi, A.G. and Rafii, M.Y. (2017). Potential genotypes with high timber volume from six species in the 1995 RRIM *Hevea* germplasm collection. Paper presented at *International Rubber Conference and IRRDB Annual Meetings 2017*, 18th -22nd October 2017, Jakarta, Indonesia.

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