**UNIVERSITI PUTRA MALAYSIA**

*GENETICS AND QUANTITATIVE TRAIT LOCI MAPPING OF BIOMASS YIELD AND QUALITY TRAITS IN MAIZE (Zea mays L.) FOR FORAGE UTILIZATION*

**NAZATUL SHIMA BINTI NAHARUDIN**

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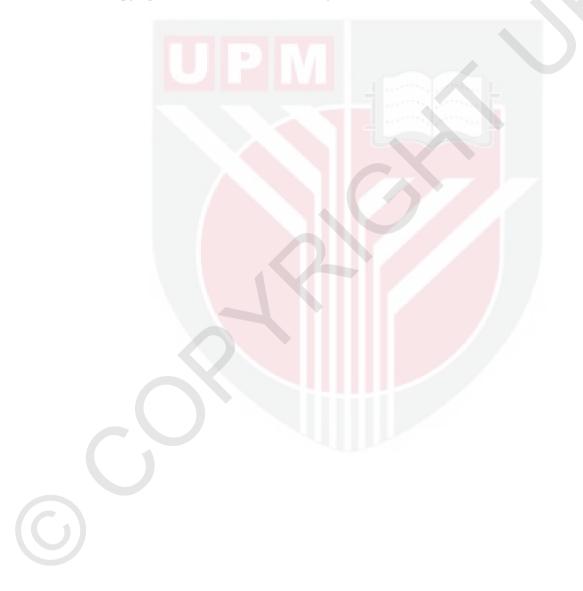
By

**NAZATUL SHIMA BINTI NAHARUDIN**

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

**June 2017**

# DEDICATION

This dissertation is dedicated to:

my backbones:

*Naharudin Musa*
*and*
*Kamarul Ashikin Muhd Piah,*

my other half:
*Nor Imran Shahabudin,*

and the apples of my eyes:
*Ammar Mujahid*
*and*
*Sumayyah Syahidah*

# GENETICS AND QUANTITATIVE TRAIT LOCI MAPPING OF BIOMASS YIELD AND QUALITY TRAITS IN MAIZE (*Zea mays* L.) FOR FORAGE UTILIZATION

By

**NAZATUL SHIMA BINTI NAHARUDIN**

**June 2017**

**Chairman** : **Professor Ghizan bin Saleh, PhD.**
**Faculty** : **Agriculture**

Genetic studies and quantitative traits loci (QTL) mapping on maize (*Zea mays* L.) for forage yield and quality traits for animal feed are lacking, especially those conducted in the tropical region. Efficient breeding and selection strategies of maize for forage utilization require sound knowledge and understanding of the genetics associated with biomass yield and forage quality. A series of experiments and analyses were conducted to elucidate important genetic parameters and to map QTLs for biomass yield and forage quality traits in maize. Two crosses between inbred lines (CML 152 × CML 383 and CML 491 × CML 331) with contrasting values for biomass yield and quality traits were made to produce two $F_2$ populations. Heterosis, inbreeding depression and broad-sense heritability of 16 biomass yield and forage quality traits were estimated from evaluation of the hybrids, the $F_2$ populations and the parents involved. In general, Cross 1 showed higher heterosis and inbreeding depression for biomass yield traits compared to Cross 2. The broad sense heritability estimates in both populations were moderate to high for plant height, dry leaf yield, protein content and acid detergent lignin, indicating that these traits can be used as the selection criteria. Predicted genetic gain from selection were found high for fresh and dry biomass yield and moderate for plant height and protein content in both populations. Correlation analysis on biomass yield and quality traits revealed that all biomass yield components were significantly correlated. For forage quality traits, moderate positive correlations were found among the traits related to cell wall composition (neutral and acid detergent fiber and acid detergent lignin contents). Moreover, these traits were also found to be moderately correlated with dry plant yield. Mapping of QTLs linked to biomass yield and forage quality traits was done on two $F_2$ mapping populations derived from CML 152 × CML 383 and CML 491 × CML 331 crosses. Out of 180 SSR markers used in screening, 61 markers were polymorphic in Cross 1 and 62 markers were polymorphic in Cross 2, which were then used to construct linkage maps. Ten linkage groups were detected in both populations with the size of 822.2 cM in Population 1 and 740.5 cM in Population 2. Data were analyzed using single marker

regression analysis and composite interval mapping analysis to detect markers and regions with significant QTLs. For biomass yield traits, eight QTLs were identified in Population 1 and 10 QTLs were detected in Population 2 based on single marker regression analysis. When combined in multiple loci model, these QTLs accounted for up to 30.41% (Population 1) and 85.89% (Population 2) of the phenotypic variation explained (PVE). QTLs on Chromosomes 1 and 8 in Population 1, and QTLs on Chromosomes 1 and 9 in Population 2 were associated with multiple traits, suggesting the presence of pleiotropic effects. Composite interval mapping analysis detected three intervals on Chromosomes 1 and 8 in Population 1 and four on Chromosomes 1, 3, 4 and 9 in Population 2 associated with biomass yield traits, some of which coincide with the markers with high PVE in single marker regression analysis. Epistatic interactions among the loci for plant height were identified, contributing 13.02% to PVE. Most of the QTLs detected for biomass yield in Population 1 were found to have dominance effects, while QTLs in Population 2 had additive effects, thus explaining the higher expressions of heterosis for biomass yield traits in Population 1 compared to Population 2. For forage quality traits, in Population 1, single marker regression detected three putative QTLs to be associated with protein content on Chromosomes 1 and 2, and eight putative QTLs for cell wall components on Chromosomes 3, 5, 8, and 9. In Population 2, four putative QTLs were detected for protein content, whereas for cell wall components, one QTL was detected for neutral detergent fiber, acid detergent fiber and acid detergent lignin contents, respectively. Composite interval mapping analysis revealed three QTLs with main effects and epistatic interactions on Chromosomes 1, 6 and 8 for acid detergent lignin in Population 1. Although no main effect QTLs were detected for neutral detergent fiber and acid detergent fiber, regions with epistatic interactions were detected for these traits. QTLs for protein content were only detected in Population 2 and none in Population 1. Some of the QTLs detected for biomass yield were also present in other inbred lines in the germplasm collection, and could be confirmed by association mapping analysis. The identified QTLs could be utilized in marker assisted breeding programs or high resolution mapping after QTL validation in various environmental condition using different populations for future forage maize improvement in the tropical region.

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

# GENETIK DAN PEMETAAN LOKUS CIRI KUANTITATIF BAGI HASIL BIOJISIM DAN KUALITI JAGUNG (*Zea mays* L.) UNTUK KEGUNAAN FORAJ

Oleh

**NAZATUL SHIMA BINTI NAHARUDIN**

**Jun 2017**

**Pengerusi : Professor Ghizan bin Saleh, PhD**
**Fakulti : Pertanian**

Kajian genetik dan pemetaan lokus ciri kuantitatif (QTL) ke atas jagung (*Zea mays* L.) untuk hasil dan kualiti foraj bagi kegunaan makanan haiwan masih ketinggalan, terutama di kawasan tropika. Program biakbaka dan pemilihan yang efektif ke atas jagung bagi kegunaan foraj memerlukan pengetahuan dan pemahaman yang lengkap mengenai kawalan genetik bagi hasil biojisim dan kualiti foraj. Beberapa kajian dan analisa telah dijalankan untuk menerangkan parameter genetik yang penting dan memetakan QTL berkaitan hasil biojisim dan beberapa kualiti foraj bagi tanaman jagung. Dua kacukan antara titisan inbred (CML 152 × CML 383 dan CML 491 × CML 331) yang berlainan bagi sifat-sifat hasil biojisim dan kualiti dilakukan sehingga membentuk dua populasi $F_2$. Nilai heterosis, kemelesetan inbred dan kebolehwarisan luas bagi 16 sifat hasil biojisim dan kualiti foraj dianggar dari analisis populasi hibrid, populasi $F_2$ dan induk masing-masing. Secara amnya, Kacukan 1 menunjukan nilai heterosis dan kemelesetan inbred yang lebih tinggi bagi hasil biojisim berbanding Kacukan 2. Nilai kebolehwarisan luas bagi kedua-dua populasi bagi sifat ketinggian tumbuhan, hasil daun kering, kandungan protein dan kandungan lignin detergen asid adalah sederhana tinggi, menunjukkan sifat-sifat ini boleh digunakan sebagai kriteria pilihan yang utama. Nilai taksiran bagi kemajuan genetik untuk hasil biojisim segar dan kering adalah tinggi manakala bagi sifat ketinggian tumbuhan dan kandungan protein adalah sederhana bagi kedua-dua populasi. Analisis korelasi antara sifat-sifat hasil biojisim dan kualiti menunjukkan yang kesemua komponen hasil biojisim berkolerasi secara signifikan. Bagi ciri kualiti foraj, kolerasi positif dan sederhana ditemukan di antara ciri-ciri berkaitan komposisi dinding sel (kandungan serat detergen neutral, serat detergen asid dan lignin detergen asid). Sifat-sifat ini juga berkolerasi secara sederhana dan positif dengan hasil biojisim kering. Pemetaan QTL yang terangkai dengan sifat hasil biojisim dan kualiti ditaksir ke atas dua populasi pemetaan $F_2$ yang diperoleh daripada kacukan CML 152 × CML 383 dan CML 491 × CML 331. Daripada 180 penanda SSR yang digunakan untuk saringan, 61 penanda SSR didapati polimorfik bagi Kacukan 1 dan 62 penanda SSR didapati polimorfik bagi

Kacukan 2. Penanda-penanda terpilih ini kemudiannya digunakan untuk membina peta rangkaian. Sepuluh kumpulan rangkaian telah dikesan bagi kedua-dua populasi, dengan saiz 822.2 cM bagi Populasi 1 dan 740.5 cM bagi Populasi 2. Data fenotip dan genotip dianalisa menggunakan regresi satu penanda dan pemetaan silang komposit untuk mengesan QTL yang signifikan. Bagi sifat hasil biojisim, lapan QTL dikenalpasti di Populasi 1 dan sepuluh QTL dikenalpasti di Populasi 2. Apabila digabung dalam model berbilang lokus, QTL tersebut menyumbangkan sehingga 30.41% (Populasi 1) dan 85.89% (Populasi 2) kepada varian fenotip. QTL di Kromosom 1 dan 8 bagi Populasi 1, dan QTL di Kromosom 1 dan 9 bagi Populasi 2 mengandungi QTL bagi pelbagai sifat, justeru menunjukkan kesan pleiotropi. Analisis pemetaan silang komposit mengesan tiga selang penanda di Kromosom 1 dan 8 bagi Populasi 1 dan empat selang penanda di Kromosom 1, 3, 4 dan 9 di Populasi 2 yang berkait dengan ciri hasil biojisim, di mana sebahagiannya merupakan penanda yang mempunyai nilai varian fenotip yang tinggi bagi analisis regresi satu penanda. Interaksi epistatik antara lokus bagi sifat ketinggian tumbuhan telah dikenalpasti dan menyumbang 13.02% kepada varian fenotip. Kebanyakan QTL yang dikesan bagi Populasi 1 mempunyai kesan dominan, manakala QTL bagi Populasi 2 mempunyai kesan aditif, justeru menerangkan nilai heterosis yang tinggi untuk hasil biojisim bagi Populasi 1 berbanding Populasi 2. Bagi ciri kualiti foraj, bagi Populasi 1, analisis regresi satu penanda mengenalpasti dua QTL putatif bagi kandungan protein dan lapan QTL putatif bagi ciri berkait komposisi dinding sel. Bagi Populasi 2, empat QTL dikenalpasti bagi kandungan protein dan satu QTL dikenalpasti untuk serat detergen neutral, serat detergen asid dan lignin detergen asid masing-masing. Analisis pemetaan silang komposit menunjukkan tiga QTL dengan kesan utama dan interaksi epistatik di Kromosom 1, 6 dan 8 bagi kandungan lignin detergen asid bagi Populasi 1. Walaupun tiada QTL kesan utama dikesan bagi kandungan serat detergen neutral dan serat detergen asid, beberapa lokus dikesan dengan interaksi epistatik bagi sifat-sifat ini. QTL bagi kandungan protein hanya dikesan bagi Populasi 2 dan tiada QTL bagi kandungan protein bagi Populasi 1. Sebahagian QTL yang berkait dengan hasil biojisim juga dikesan di titisan inbred lain di dalam koleksi germplasma dan boleh dipastikan melalui analisis pemetaan perkaitan. Semua QTL yang dikesan boleh digunakan untuk program biakbaka berbantu penanda atau program pemetaan beresolusi tinggi selepas pengesahan QTL dijalankan di persekitaran yang berbeza ke atas populasi yang berbeza, untuk program pembiakbakaan jagung foraj di kawasan tropika.

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I certify that a Thesis Examination Committee has met on 15 June 2017 to conduct the final examination of Nazatul Shima binti Naharudin on her thesis entitled "Genetics and Quantitative Trait Loci Mapping of Biomass Yield and Quality Traits in Maize (*Zea mays* L.) for Forage Utilization" 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.

Members of the Thesis Examination Committee were as follows:

**Halimi bin Mohd Saud, PhD**
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

**Mohd Rafii bin Yusop, PhD**
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Internal Examiner)

**Mohamad bin Osman, PhD**
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

**Irwin L. Goldman, PhD**
Professor
University of Wisconsin
United States
(External Examiner)

**NOR AINI AB. SHUKOR, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 August 2017

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:

**Ghizan Bin Saleh, PhD**
Professor
Faculty of Agricuture
Universiti Putra Malaysia
(Chairman)

**Nur Ashikin Psyquay Abdullah, PhD**
Associate Professor
Faculty of Agricuture
Universiti Putra Malaysia
(Member)

**Maheran binti Abd Aziz, PhD**
Associate Professor
Faculty of Agricuture
Universiti Putra Malaysia
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| Signature: | |
| Name of Chairman of Supervisory Committee: | Professor Dr. Ghizan Bin Saleh |

| | |
|---|---|
| Signature: | |
| Name of Member of Supervisory Committee: | Associate Professor Dr. Nur Ashikin Psyquay Abdullah |

| | |
|---|---|
| Signature: | |
| Name of Member of Supervisory Committee: | Associate Professor Dr. Maheran binti Abd Aziz |

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| | |
|---|---|
| ADF | Acid detergent fiber |
| ADL | Acid detergent lignin |
| AFLP | Amplified fragment length polymorphism |
| ANOVA | Analysis of variance |
| Bin | A sub-region on corn chromosome |
| bp | Base pairs |
| CIMMYT | Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center) |
| cM | centiMorgan |
| CIM | Composite interval mapping |
| CTAB | Cetyl – trimethylammonium bromide |
| DH | Doubled haploid |
| DNA | Deoxyribonucleic acid |
| dNTP | 2'-Deoxyribonucleic acid 5'-triphosphate |
| EDTA | Ethylenediaminetetra-acetic acid |
| FAO | Food and Agriculture Organization of the United Nations |
| GML | Ground magnesium limestone |
| $h_B^2$ | Broad-sense heritability |
| ISSR | Inter simple sequence repeats |
| LOD | Likelihood of odds |
| MAS | Marker assisted selection |
| MgCl | Magnesium chloride |
| NaCl | Sodium chloride |
| NaOAc | Sodium acetate |

| | |
|---|---|
| NDF | Neutral detergent fiber |
| NIRS | Near infra-red spectrometer |
| ng | nanogram |
| PCR | Polymerase chain reaction |
| QTL | Quantitative trait loci |
| r | Correlation coefficient |
| RAPD | Random amplified polymorphic DNA |
| RCBD | Randomized complete block design |
| RFLP | Restriction fragment length polymorphism |
| RIL | Recombinant inbred lines |
| RNA | Ribonucleic acid |
| rpm | Revolution per minute |
| SMA | Single marker analysis |
| SNP | Single nucleotide polymorphisms |
| SSR | Simple sequence repeats |
| TBE | Tris-borate/EDTA |
| Taq | *Thermus aquaticus* |
| TE | Tris EDTA buffer |

# CHAPTER 1

## INTRODUCTION

Maize (*Zea mays* L.) is one of the important cereal crops planted in Malaysia, with the current planting area of 28,000 hectares in 2013-2014 (FAOSTAT, 2015). Although the domestic consumption and demand of fresh and processed maize increase every year both for food and feed industries, the local maize production still lags behind. Apart from sweet corn and grain maize breeding programs, which are the two main focuses in Malaysia's maize breeding scenario, the development of forage maize with high biomass yield, good nutritional properties and digestibility for the utilization of livestock feed and silage has become a new interest in the current maize breeding objectives. Besides the common use as food for human consumption, maize is also a preferred forage crop to be utilized as fresh fodder, or to be ensiled as quality animal feed, and this is a wide and common practice in developed countries such as the US, EU and Australia (Farnham *et al.,* 2003). It is accepted that a high quality forage or enriched silage for animal feed can increase the quality and quantity of meat production. Nevertheless, in Malaysia, animal feed are ensiled and processed from either forage crop such as grass, grass-related species and Napier (Chin and Idris, 2000; Chin, 2001), or agriculture wastes and by-products such as rice straw and oil palm fronds (Abdalla *et al.,* 1998; Abdalla *et al.,* 2001). There is not much focus given on developing specific maize variety for animal feed purposes with the aim of increasing its biomass yield and feed quality aspects.

This study was undertaken as a part of an extensive silage maize breeding program, with the ultimate goal of producing high yielding and high quality hybrid varieties for forage utilization and silage production. It is primarily pivotal to understand and elucidate the genetics biomass yield and forage quality traits in maize for forage utilization, since these traits do not get much attention in maize local breeding programs. Particularly for forage maize, the biomass yield and forage quality traits are determined by the interaction between their genetic constitutions with the environment, thus, the phenotypic measurements alone do not reveal the real genetic potential of the plant. Furthermore, the genetics of yield and yield related traits of maize for silage production has not been studied particularly in the tropical region. Understanding the genetic control of these traits could be achieved by obtaining important genetic parameters, such as heritability, heterosis and inbreeding depression, gain from selection, and the phenotypic and genetic correlations amongst the traits. Knowledge of such information is crucial in comprehending the overall genetic control for these complex traits.

The incorporation of molecular markers approach in the breeding program serves as a catalyst to expedite the selection process, together with phenotypic-based selection method. Molecular markers are useful tools in Marker Assisted Breeding (MAB) programs because they enable the association of DNA markers with the traits of interest. By screening and mapping markers covering the genome, through statistical

analysis, markers that are tightly linked to favorable traits can be identified. Mapping and identification of quantitative traits loci (QTL) related to agronomically important traits that usually have complex genetic control is a helpful strategy that can accelerate the selection process of quantitative traits in breeding populations. With the use of molecular markers, demarcation of loci for important traits related to biomass yield, protein content and digestibility can be made and can further be utilized in molecular assisted breeding. QTL mapping provides useful information for breeding programs since it allows estimation of genomic locations and genetic effects of chromosomal regions tagged by molecular markers that are associated with the expression of any traits. The outcome for this study is detailed information on association of markers and yield related traits with defined QTL regions along the chromosomes. The markers associated to the traits can be used in marker assisted breeding programs for the development of high yielding and high quality forage maize varieties.

It is hopeful that from this study, the genetic control of biomass yield and quality traits in maize for forage utilization can be elucidated and understood. Alleles for highly digestible cell wall were reduced or perhaps lost by genetic drift after generations of selections for stalk standability and breakage resistance (Barriere *et al.,* (2005), which resulted in lignified stems. The estimates for phenotypic and genetic correlation analyses of traits involved in biomass yield and quality can also provide direction of selection in breeding programs. Combined with heritability estimates, effective genetic advance for these traits can be made to improve maize for forage utilization. Molecular analysis particularly QTL mapping can identify chromosomal regions associated with biomass yield and quality traits. As a preliminary mapping study of forage traits in maize in tropical environment, this can open more in depth analyses on fine mapping up to gene isolation and identification. Molecular markers that are tightly linked to the traits analyzed can be exploited in MAB programs.

Therefore, the general objectives for this study were:

1. To understand the genetics of biomass yield and quality traits in maize for forage utilization
2. To identify informative microsatellite markers and QTLs highly associated with biomass yield and quality traits for the purpose of marker assisted breeding programs.

In order to achieve the general objectives, several specific objectives were set. These objectives were:

1. To estimate genetic parameters of yield and quality trait of maize for forage utilization,
2. To map quantitative traits loci for biomass yield and yield related components in maize for forage utilization,
3. To map quantitative trait loci for quality and quality related components in maize for forage utilization,

4. To compare the genetic control of biomass yield and quality traits in two populations based on QTL analyses, and
5. To check presence of QTLs associated with biomass yield and protein content in maize inbred lines.

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