WOOD DENSITY AND CLEAVED AMPLIFIED POLYMORPHIC SEQUENCE (CAPS) OF *Acacia* SPP.

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Master of Science
2012
WOOD DENSITY AND CLEAVED AMPLIFIED POLYMORPHIC SEQUENCE (CAPs) OF ACACIA SPP.

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Thesis is submitted
In fulfillment of requirements for degree of Master Science

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2012
ACKNOWLEDGEMENT

Praise and thanks to ALLAH, Most Gracious, Most Merciful, for giving me the strength and perseverance to complete this thesis. May ALLAH accept this humble effort of mine.

I would sincerely like to express my heartfelt gratitude to my supervisors Associate Professor Dr Ismail Jusoh and Dr Ho Wei Seng for providing me the opportunity to undertake my Masters study at UNIMAS, their supervision and support for this research is greatly appreciated. Over the years they had guided me with great patience and enthusiasm and given me invaluable advice throughout the course of this study.

In addition, the accomplishment of CAPs test must be credited to Professor Wickneswari Ratnam of UKM, for the constructive discussions and suggestions as well as giving me the opportunity to conduct my molecular research at the Forest Genetics Laboratory in UKM, Bangi. To Miss Christina Yong of Forest Genetics Laboratory in UKM whom shared invaluable information which enable me to design my primer pairs. Many thanks must also go to Mr Rizan Abdullah and Tuan Haji Kurni Taha for their assistance with my laboratory test. I would like to extend my appreciation to Borneo Tree Seeds and Seedling Supplies Sdn Bhd and Daiken plantation for allowing me to obtain my samples from their plantations. A special thanks also goes to the administrations of Centre of Graduate Studies and Faculty of Resource Science and Technology. The financial support provided by UNIMAS, Zamalah Postgraduate Scholarship is gratefully acknowledged.
All my fellow colleagues in UNIMAS and UKM have been very supportive and created a pleasant research environment, for which I am truly grateful. Particularly I wish to thank, Angela Henry, Juraidah Salimun, Jafaruddin Ali, Lau Ee Ting, Lee Hwei Huih, Christina Yong, Sukganah and Mee Siing for sharing invaluable information, their laughter and constructive discussions from time to time. Many other individuals, more than this small page can contain, have brought me much needed encouragement and support. Thank you for making this an excellent place and time for my research.

Finally, to my family, to whom this thesis is dedicated. I am indebted to my father for his perpetual care and support, and am also grateful to my siblings and parents-in-law who are ever encouraging. To my hero, my husband, Fauzan Sahdi, I cannot thank you enough for your unconditional love, motivations, patience and support. The arrival of baby Ayesha added several sleepless nights, but also brought along bundles of joy and happiness to our family. This fuelled me with much motivation and encouragement. For your love, I am truly overwhelmed with gratitude.
ABSTRACT

(Developing genetically improved plant material of the best adapted plantation species will improve yield and wood quality from the plantations. To assess the quality of *Acacia* spp. wood available, an understanding of wood properties and genetic analysis of wood quality traits should be done by combining morphological and molecular techniques. Fifty six samples of *Acacia* hybrid, *Acacia mangium* Superbulk and *Acacia auriculiformis* were analyzed with cleaved amplified polymorphic sequence (CAPs) markers and the specific gravity of all samples were. The main objectives of this study were to determine and compare specific gravity of the selected *Acacia* species from different populations and between the species and to analyze polymorphism of gene (*CesA1*) using cleaved amplified polymorphic sequence (CAPs).) Determination of specific gravity was carried out in accordance to the American Society for Testing and Material (ASTM) Standard D2395 method B. Two specific primer pairs namely CS1 and CS2 were designed based on the full length cDNA of *CesA1* from *A. mangium*. Five restriction enzymes, i.e. *Alul*, *Ddel*, *DpnI*, *NlaIV* and *BslI* which demonstrated the presence of restriction sites and scorable bands were selected for CAPs analysis. The results showed that specific gravity varies with populations and species. *A. auriculiformis* recorded the highest specific gravity value with 0.55, followed by *Acacia* hybrid and *A. mangium* Superbulk with 0.50 and 0.44, respectively. Variation of CAPs profile could be observed from the digestion of *CesA1* gene region of *A. mangium* Superbulk from BTSSSB by using *Ddel* restriction enzymes. Sample SB5 produced a unique banding profile in which an extra fragment (or InDel polymorphism) of approximately 200 bp was detected. Though InDel polymorphism was detected in this sample, specific gravity data did not correlate with the molecular variation of *CesA1* gene in *A. mangium* Superbulk in this study.
Further research should be done using more restriction enzymes and more samples of the
Acacia spp. should be used.
KETUMPATAN KAYU DAN JUJUKAN POLIMORFISME TERAMPLIFIKASI
TERPOTONG (CAPs) ACACIA SPP.

ABSTRAK

Pembangunan baka tanaman yang telah dimodifikasi secara genetik bagi spesies perladangan boleh meningkatkan hasil serta kualiti kayu dari ladang hutan. Untuk menilai kualiti kayu spesies Acacia yang ada, pemahaman terhadap sifat-sifat kayu dan analisis genetik ciri-ciri mutu kayu perlu dilakukan dengan menggabungkan kajian morfologi dan molekul. Lima puluh enam sampel Acacia hybrid, Acacia mangium Superbulk dan Acacia auriculiformis dianalisis dengan penanda jujukan polimorfisme teramplifikasi terpotong (CAPs) dan graviti spesifik setiap sampel ditentukan. Objektif utama kajian ini adalah untuk menentukan dan membandingkan gravity spesifik di antara spesies acacia di antara populasi yang berbeza serta menganalisis polimorphism gen (CesAl) menggunakan CAPs. Penentuan graviti spesifik dijalankan berdasarkan kaedah Persatuan Amerika untuk Ujian dan Bahan (ASTM) Standard D2395 metod B. Dua pasangan primer khusus CS1 dan CS2 telah direka berdasarkan cDNA penuh CesAl dari Acacia mangium. Lima enzim sekatan Alul, Ddel, DpnI, NlaIV dan Bsll yang menunjukkan kehadiran tapak sekatan dan jalur yang boleh skor dipilih untuk analisis CAPs. Keputusan menunjukkan graviti spesifik berbeza mengikut populasi dan spesies. Acacia auriculiformis menunjukkan ia mempunyai spesifik graviti tertinggi dengan nilai 0.55, diikuti Acacia hybrid dan A. mangium Superbulk dengan masing-masing mencatatkan nilai 0.50 dan 0.44. Variasi di dalam profil CAPs boleh diperhatikan dari pencernaan rantau CesAl gen A. mangium Superbulk dari BTSSSB dengan menggunakan enzim sekatan Ddel. Sampel SB5 telah menghasilkan profil jalur yang unik di mana serpihan tambahan (polimorfisme InDel) kira-kira 200bp telah dikesan. Walaupun polimorfisme InDel dikesan dalam sampel ini, data graviti spesifik tidak berkorelasi dengan
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CHAPTER ONE

INTRODUCTION

1.1 General Background

In general, genetic studies have shown that most wood physical properties have high heritability for examples in pines and hardwood (Stanger et al., 2002), which suggests that selection and breeding for these properties should be successful. Zobel and Jett (1995) stated that wood improvement is most needed for trees that are grown as exotics in the tropics and subtropics. The advantages of exotics are rapid growth and a young harvestable age, but generally, they have poor form and a high proportion of juvenile wood. Much of the recent expansion in plantation forestry is occurring in the tropics and subtropics, a number of new species are being tested such as *Acacia mangium*, *Paraserianthsis falcataaria*, *Acacia auriculiformis*, *Tectona grandis*, *Swietenia macrophylla*, and *Gmelina arborea* (FAO, 2002). Developing genetically improved plant material of the best adapted plantation species will improve yield and wood quality from the plantations. According to Evans and Turnbull (2004) in other tropical countries the availability of good quality planting stock is one of the major limitations to plantation development.

Wood density is an important indicator of wood quality (Haygreen and Bowyer, 2003) for lumber and pulp yield and thus is of economic importance to forest industry. It is the properties that have been most widely studied genetically, although it is a complex property dependent on a wide variety of anatomical variables (Nylinder, 1965). Wood density is expressed in terms of wood specific gravity, defined as the ratio of wood substance to pure
water at 4°C (Panshin and DeZeeuw, 1980). Wood specific gravity is not a single property but it is a trait influenced by the combination of wood properties namely fibre length, diameter and wall thickness. The strong relationship of specific gravity to mechanical properties, fiber yield and other properties significant to the end use of forest products and the simplicity of its determination, makes it simple and good indicators of wood. Genetic control of most wood properties is moderate to very strong and despite the apparent genetic control of wood properties that make up wood density, their combine effect, that is, wood density shows strong heritability (Zobel and Jett, 1995). As a result there is a large genetic variation in basic density between trees which makes it possible to select trees with a higher growth rate and moderately high density for breeding (Saranpää, 2003).

Cleaved Amplified Polymorphic sequence (CAPs), a technique to identify polymorphisms at a particular locus used to characterize the Acacia spp. at the molecular level. It is a technique where DNA fragments are amplified using a specific primer then are digested by using restriction enzymes to reveal sequence polymorphisms. In which the polymorphisms of the sequence is an outcome from cutting the products in different places and the variants are revealed as length differences when running the reactions on agarose gels (Muchugi et al., 2008). CAPs marker is similar to RFLP marker however; PCR is used in CAPs to detect restriction site polymorphisms instead of DNA blot hybridization and sequencing is not necessarily required. CAPs markers require only small quantities of genomic DNA based on PCR for detection. Typically, a single leaf will provide enough DNA for analysis with multiple and CAPs markers can be easily assayed using standard agarose gel electrophoresis (Giraudat et al., 1999). CAPs that possess the properties of being co-dominant allows the differentiation of heterogoggles and homozygotes. CesA is the key enzyme
involved in the regulation of cellulose biosynthesis pathway (Campbell et al., 1997). They are heritable and important in determining the variability of the wood. Hence, it provides a greater impact on the design of future genetic improvement strategies in the production of wood with better quality.

It is widely recognized that plantation forest is more productive than natural forest and provides higher economic benefits as experienced in many places (Sedjo, 2001). The main aim of this study was to assess the quality of *Acacia* spp wood available for the pulp, paper and board industries through an understanding of wood properties and genetic analyses of wood quality traits. These combines morphological and molecular studies on *Acacia* spp. in order to get insight into the understanding of wood quality and to obtain information on genotypic variation within *Acacia* species. This study includes comparing selected anatomical properties and mechanical properties of *Acacia* spp. grown in three different locations. Results of this study will contribute and lead to more efficient breeding methods for wood characters; estimate of genetic correlations involving wood characters, and perhaps also an improvement in the quality of wood available to meet future demand.
1.2 Objectives

The objectives of this study were:

(1) To determine wood density of *Acacia* species from different populations, namely *Acacia* hybrid, and *Acacia mangium* Superbulk.

(2) To evaluate and compare wood density between *Acacia* hybrid, *A. auriculiformis* and *A. mangium* Superbulk.

(3) To design primers which amplify the CesA1 region of *Acacia* spp..

(4) To analyze polymorphism of gene (CesA1) related to wood density of *Acacia* spp. using Cleaved Amplified Polymorphic sequence (CAPs).
CHAPTER TWO

LITERATURE REVIEW

2.1 Background

Forests are known as an important source in trade and industrials activities. According to International Union for Conservation of Nature (IUCN), in 1980 alone the value of forest product annually exceeded 115 500 million US dollar and in the period of 2003 to 2007, wood removal valued just over 100 billion dollar annually (FAO, 2010). For the production of wood and non-wood forest products almost 1.2 billion hectares are managed primarily for it and the forest and trees which are planted for the many purposes make up an estimated 7 percent of the total forest area (FAO, 2010). The demands for timbers are expected to increase as the world population increases, however most forest products are limited to certain species of trees. Thus, in vast logging area, it can be expected that one or two valuable tree species are facing extinction. If steps have not been taken, it is anticipated that the timber industries will face difficulties in obtaining quality raw material for local and international market.

One way to address the shortage of timber from natural forest is to establish forest plantations. Forest plantations are becoming an increasingly important resource worldwide. FAO (1998) defined forest plantations as “forest stands established by planting and/or seeding in the process of afforestation or reforestation. They are either introduced species (planted stands), or intensively managed stands of indigenous species, which meet all the following criteria: one or two species at planting, even age class, regular spacing”. Shaharuddin (2001) pointed out, one crucial aspect for successful plantation species is to have...
trees either indigenous or exotic that is fast growing and could generate immediate attractive return. It is important to determine the most suitable species that would ultimately generate revenue from commercial establishment of forest plantations and *Acacia* spp. is one of the most promising species for plantation. To supplement future supplies and demands of wood and wood fibres, the State Government of Sarawak is committed to establish about 1 million ha of planted forests by the year 2020 and *Acacia mangium* will be the main species for forest plantation.

In the last decades, the focus of most tree breeding programs was to increase stem volume through genetic selection for height and diameter growth (Zobel and Talbert, 1984). According to Kellog (1989), wood and fibre quality has become one of the major concerns in the forest products industry throughout the world seeing that the managed plantations have steadily increased and forest management have moved in short rotations. Tree breeders now realized that wood quantity and quality could not be treated as independent factors. The improvement of wood quality should form an integral part of most breeding programs (Kellogg, 1986). Zobel and Van Buijtenen (1989) noted that wood quality traits should be included in any of tree improvement programs where wood is to be the end product as important consideration. The genomic study of wood quality plays an important role in incorporating wood quality traits into tree breeding programs (Zhang and Morgenstern, 1995).

### 2.2 *Acacia* species

*Acacia* is a widespread genus that occurs naturally on all continents, except Europe and Antarctica (Maslin, 2001). Over 1200 species have been described, 955 of which are