

APPLICATIONS OF GENOMICS TO *Acacia mangium* PLUS TREES (SUPERBULK) AND *Acacia* HYBRID TREE IMPROVEMENT FOR WOOD QUALITY

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Acacia mangium superbulk and *Acacia* hybrid genetics and genomics studies are getting much attention because of its economical and ecological importance as well as scientific interest. Genomic information including genome sequence and expressed sequence tags (ESTs) is essential to understand the genes that control traits that are relevant to breeding and tree improvement programmes. The objectives of this study were to (a) determine the genetic diversity of *Acacia mangium* superbulk using expressed sequence tag derived short sequence repeat (EST-SSR) markers, and (b) analyse polymorphism of gene (i.e. cellulose synthase) related to wood density of *Acacia* spp using Cleaved Amplified Polymorphic sequence (CAPS). EST-SSRs are embedded in functional gene sequences and the markers may lead to the identification of genes controlling these traits [4]. Although EST-SSR markers are less polymorphic than genomic SSR markers, they are more useful in the assessment of functional diversity and comparative mapping while genomic SSRs are superior for fingerprinting and variety identification studies (Varshney *et al.*, 2005). In the present study, 93 fresh young leaf samples were collected from 3 different areas, namely Similajau (Borneo Tree Seeds Seedlings Supply Sdn Bhd.-BTSSSB), Bintulu (DAIKEN) and Kota Samarahan (UNIMAS arboretum) and 13 EST-SSR markers were chosen for determining the genetic diversity of *A. mangium* superbulk. Total genomic DNA was isolated from fresh leaves of *A. mangium* superbulk using a modified CTAB method. Fragment analysis was conducted by using ABI PRISM[®] 3100 Genetic Analyzer and Genemapper[™] Version 4.0 software (Applied Biosystems, USA). The genotype data was then analysed using PowerMarker software to determine the genetic diversity of *A. mangium* superbulk. *A. mangium* superbulk collected from BTSSSB showed the highest level of genetic diversity (N = 40; H_e = 0.474; PIC = 0.432) compared to Daiken (N = 40; H_e = 0.458; PIC = 0.429) and UNIMAS Arboretum (N = 13; H_e = 0.364; PIC = 0.333) with an average of 0.432 and 0.398 for H_e and PIC, respectively detected in the present study. This result was comparable to the genetic diversity estimated for 11 populations of *A. mangium* in Pacific regions with the mean expected heterozygosity (H_e) of 0.081 as revealed by using isozyme markers [6]. *A. mangium* superbulk is the improved material or second generation of *A. mangium* whose properties and characters have been improved through many years of selected planting by BTSSSB in Similajau, Sarawak. The original seed sources were obtained from the CSIRO first generation seedlings and seed orchards of *A. mangium* in North Queensland.

Cleaved Amplified Polymorphic sequence (CAPS) markers are amplified by PCR, the amplified DNA will be cleaved with the carefully chosen restriction enzyme such as that through *in silico* restriction analysis and the cleaved products are examined on agarose gels [3]. CAPS that possess the properties of being co-dominant allow the differentiation of heterozygotes and homozygotes. CesA is the key enzyme involved in the regulation of cellulose biosynthesis pathway [1]. 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. We examined the molecular diversity of CesA generated through PCR amplification followed by restriction enzyme analysis from 10 selected *Acacia* spp samples from 3 different locations, i.e. BTSSSB, DIAKEN and UNIMAS arboretum. Two specific primer pairs were designed based on the full length cDNA of *CesA1* from *A. mangium*. The amplified PCR products were approximately 2.4 kb and 1.4 kb respectively and 5 restriction enzymes, i.e. AluI, DdeI, DpnI, NlaIV and BslI which demonstrated the presence of restriction sites and scorable bands were selected for CAPS analysis. The digestion of *CesA1* gene region of the *Acacia* spp using AluI, DpnI, NlaIV and BslI did not show any variation in the restriction pattern. This indicates that the amplified *CesA1* gene region in *Acacia* spp is well conserved in the present study. However, variation of CAPS profile could be observed from the digestion of *CesA1* gene region of *A. mangium* superbulk from BTSSSB by using DdeI restriction enzymes. Sample SB5 produced a unique banding profile in which an extra fragment (or InDel polymorphism) of approximately 200 bp was detected in the present study. This fragment was absent in other samples. Though InDel polymorphism was detected in this sample, wood density (specific gravity) data did not correlate with the molecular variation of *CesA1* gene in *A. mangium* superbulk in this study. As Joshi (2003) reported, even a single base pair mutation in the coding region of the *CesA* gene in *Arabidopsis* will impact the process of cellulose biosynthesis. This is consistent with the findings that *irx3* (irregular xylem 3) point mutation in *AtCesA7* show a defect in xylem secondary cell wall formation which lead to the weakened walls of the tracheary elements and later collapse upon themselves [7,8]. Thus, this finding needs to be verified in the near future.

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