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GENETIC LINKAGE AND QUANTITATIVE TRAIT LOCI (QTL) MAPPING IN *HEVEA* LATEX-TIMBER CLONES

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By

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Hevea brasiliensis or Rubber is the second most important commodity crop in Malaysia after Oil Palm. This crop has been extensively cultivated in South-east Asia region but has its origin from the Amazon Basin of South America. This crop is propagated commercially by hand pollination and budgrafting using selected parental clones and has caused inbreeding depression as seen in the low fruit set per pollination (average of ten seeds per 100 flowers pollinated). Estimation of genetic variability became necessary with the incorporation of the new prospected materials from Brazil compared to the classical cultivated clones. Thus molecular markers were incorporated into the studies on *Hevea*, since no distinct morphological traits exist between *H. brasiliensis* clones. In this study, comparisons of genetic linkage maps between two *Hevea* populations were conducted.

Genetic linkage maps of clones RRIM 937, RRIM 600, PB 5/51 and IAN 873 were constructed with molecular markers generated using the Amplified Fragment Length Polymorphism (AFLP) technique. These maps covered 7.2-13% of the estimated genome length which is between 2000 cM to 3000 cM. Genetic maps for RRIM 937 and RRIM 600 showed that the maps did not have the basic number of linkage groups of 18. One explanation is that both clones are closely related in which RRIM 600 the re-current parent is both paternal and maternal. Thus molecular markers that are generated were mostly monomorphic and uninformative in genetic map construction. Meanwhile, genetic maps for clones PB 5/51 and IAN 873 spans 18 and 17 linkage groups respectively. These two clones are quite distantly related i.e IAN 873 has a Brazilian clone, FA 1717 for a paternal parent. Nevertheless, the amount of markers generated for this population was not enough to create a dense coverage for the maps. Due to the paucity of the markers generated for all four clones, the majority of the markers were placed on Group 1 whilst the other linkage groups had 2-4 markers each and most of them were located near the centromere.

Locations of two Quantitative Trait Loci (QTLs) associated to susceptibility to *Corynespora cassiicola* were on linkage group 1 of clone IAN 873. None were found on the PB 5/51 genetic map. The Logarithim of Odds (LOD) score for these QTLs were insignificant due to the small number of progenies used in the disease screening. Using the interval mapping approach, a major QTL (*Cc1-1ian*) was located on Group 1 of IAN 873, which explained 10.1% of the phenotypic variation and a minor QTL (*Cc1-2ian*) was located on the same linkage group, explaining 5.2% of the phenotypic variation. The

subsequent Multiple-QTL Model (MQM) analysis was also conducted and did not reveal any new QTLs on any other linkage groups but was able to pin-point the positions of the QTLs closer to markers P2E2M13205 and P2E2M13198/ P2E2M13220 for *Cc1-1ian* and marker P2E2M13192 for *Cc1-2ian*.

The analysis of the differential display patterns obtained from 30 primer combinations had produced 14 specific cDNA fragments and it appears that more genes were expressed in both tolerant and susceptible clones after 72 h of inoculation. Genes are being turned on upon infection and the tolerant clones seem to have more genes expressed at this stage and cDNAs extracted from the Differential Display Reverse Transferase (DDRT) gels had high homology with stress-related genes. Differential display products excised from *Hevea* clones that were susceptible to *C. cassiicola* had strong homology to *Populus* x *canadensis* mRNA for putative histidine-containing phosphotransfer protein 2 (hpt2 gene) which is involved with osmotic stress and cell growth, disease resistance protein (RPM1) found in castor beans (*Ricinus communis*), *Alternanthera philoxeroides* (Aligator weed) salinity-induced protein (SI10) and EST from severe drought-stressed *Populus* leaves.

It is possible that in *Hevea*, the gene related to osmotic stress is turned off upon release of cassiicolin, resulting in the collapse of the cells and these genes could be induced locally rather than systematically as the colonization of the pathogen only occurs when

cassiicolin is present. A large portion of the cDNA extracted from the DDRT gel (82%) lacked any similarity to any known proteins (data not shown). They were classified either as transcripts lacking similarity to any known sequences or as transcripts not producing any hits. These genes of unknown function could probably be transcripts that are specifically expressed in leaves during stress/defense related conditions.

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sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

GENETIC LINKAGE AND QUANTITATIVE TRAIT LOCI (QTL) MAPPING IN *HEVEA* LATEX-TIMBER CLONES

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Hevea brasiliensis atau pokok getah merupakan tanaman komoditi yang kedua pentingnya di Malaysia selepas kelapa sawit. Tanaman ini ditanam dengan meluas di kawasan Asia Tenggara tetapi adalah berasal dari Lembah Amazon di benua Amerika Selatan. Pokok getah dibiakkan secara komersial menggunakan kaedah pendebungaan tangan dan dengan teknik cantuman mata tunas dengan menggunakan klon-klon terpilih. Kaedah ini menyebabkan tekanan pembiakbakaan yang dapat dilihat dengan kadar berputik yang rendah bagi setiap hasil pendebungaan (purata sepuluh biji benih untuk setiap 100 pendebungaan). Penilaian keragaman genetik adalah perlu dengan penerapan bahan genetik baru dari Brazil. Oleh itu petanda molekular diterapkan dalam kajian berkenaan *Hevea*, memandangkan tiada ciri morfologi yang berbeza. Dalam kajian ini, perbandingan antara peta rangkaian genetik terhadap dua populasi telah dijalankan.

Peta rangkaian genetik bagi klon RRIM 937, RRIM 600, PB 5/51 dan IAN 873 telah dibina menggunakan petanda molekular yang dijana oleh teknik Amplified Fragment Length

Polymorphism (AFLP). Peta-peta tersebut hanya melitupi 7.2-13% dari genome yang dianggarkan merangkumi di antara 2000 cM ke 3000 cM. Peta genetic bagi RRIM 937 dan RRIM 600 menunjukkan bahawa peta-peta tersebut tidak mempunyai bilangan asas kumpulan pautan sebanyak 18. Salah satu dari sebab ialah kerana kedua-dua klon berkait rapat di mana klon RRIM 600 adalah induk yang berulang. Oleh itu petanda molekular yang dijana adalah monomorfik anda tidak dapat diguna pakai untuk membina peta genetik. Manakala, peta-peta genetik bagi klon PB 5/51 dan IAN 873 merangkumi 18 dan 16 kumpulan pautan setiap satu. Ini adalah kerana klon IAN 873 mempunyai induk jantan, FA 1717 dari Brazil.

Walaubagaimanapun, jumlah petanda molekular yang dijana tidak mencukupi untuk membina peta yang padat. Di sebabkan kekurangan petanda molekular yang dijana untuk keempat-empat klon, majoriti petanda tersebut di letakkan pada Kumpulan 1 sementara kumpulan yang lain hanya ada 2-4 petanda setiap-satu dan kebanyakkan nya terletak di Bahagian centromere.

Kedudukan dua Quantitaive Trait Loci (QTL) yang berhubung kait dengan keadaan mudah mendapat penyakit dari *Corynespora cassiicola* terdapat pada kumpulan pautan 1 klon IAN 873 dan tiada pada peta genetik klon PB 5/51. Skor LOD bagi QTL tersebut tidak ketara/significant memandangkan bilangan progeni yang digunakan adalah kecil. Dengan menggunakan “interval mapping approach”, QTL yang mempunyai pengaruh

besar (*Cc1-1ian*) ditempatkan pada kumpulan 1 klon IAN 873 dengan pengaruh variasi sebanyak 10.1% dan satu QTL yang mempunyai pengaruh kecil (*Cc1-2ian*) terletak pada kumpulan yang sama dengan pengaruh variasi sebanyak 5.2% sahaja. Multiple-QTL model (MQM) juga digunakan tetapi tidak menyerlahkan QTL yang baru di mana-mana kumpulan pautan yang lain, malahan model ini telah menjuruskan kedudukan QTL-QTL tersebut ke petanda P2E2M13205 dan P2E2M13198/ P2E2M13220 untuk *Cc1-1ian* and petanda P2E2M13192 untuk *Cc1-2ian*.

Analisa corak differential display yang didapati dengan menggunakan 30 kombinasi primer telah menjana 14 cebisan cDNA dan didapati bahawa kebanyak gen menghasilkan isyarat pada klon yang tahan penyakit dan yang rentan dalam masa 72 j selepas inokulasi. Ini adalah satu pemerhatian yang menarik kerana diperhatikan gen-gen mengeluarkan isyarat apabila diserang penyakit dan klon yang toleran didapati mempunyai lebih gen tersebut pada tahap ini. Didapati bahawa cDNA dari gel Differential Display Reverse Transferase (DDRT) mempunyai homologi yang tinggi kepada gen berkaitan tekanan/stress. Hasil differential display yang dipencilkan dari klon *Hevea* yang mudah mendapat penyakit dari *C. cassiicola* ada homologi yang kuat kepada mRNA bagi *Populus* x *canadensis* yang berperanan dalam tekanan osmosis dan pertumbuhan sel, protin tahan penyakit (RPM1) yang didapati dalam castor beans (*Ricinus communis*), *Alternanthera philoxeroides* (Aligator weed) protin aruhan-kemasinan (SI10) and EST dari daun *Populus* yang melalui tekanan kemarau yang teruk.

Adalah berkemungkinan bahawa pada *Hevea*, gen yang berkaitan tekanan osmosis dipadam apabila toxin cassiicolin dikesan dalam sistem pokok, menyebabkan sel rosak dan gen-gen ini dipengaruhi secara setempat dan bukan secara sistematik kerana penjajahan pathogen hanya berlaku apabila terdapat cassiicolin. Kebanyakan cDNA yang dipencilkan dari gel DDRT (82%) tidak mempunyai persamaan kepada mana-mana protein. cDNA ini di kelaskan sebagai transkrip yang tiada apa-apa persamaan. Gen-gen yang dikenal pasti tiada apa-apa fungsi berkemungkinan adalah transkrip yang di "express"kan khas dalam daun semasa dikenakan tekanan/stress atau dalam keadaan pertahanan.

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I certify that a Thesis Examination Committee has met on the 23rd of August 2011 to conduct the final examination of Safiah bt. Atan on her thesis entitled "Genetic Linkage and Quantitative Trait Loci (QTL) Mapping in *Hevea* Latex-Timber Clones" 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.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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SAFIAH BT. ATAN

Date: 28th August 2011

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