MORPHOLOGICAL AND MOLECULAR ASSESSMENT OF DURIAN GERMPLASM IN MALAYSIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to:

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Genetic resource management is essential for the continuity of improvement and production of durian. It involves maintaining associated data, and characterising and assaying highly heritable morphological and molecular traits for taxonomic study, genetic improvement, quality assurance and management purposes. The objective of this study was to determine the genetic variations of durian germplasm in Malaysia, based on morphological traits and PCR-RFLP on chloroplast DNA and ribosomal DNA regions.

For study of genetic variation based on morphological traits, a total of 65 durian accessions, consisting of 60 cultivated clones and five wild species were compared. A total of 12 distinct clusters were identified separating the accessions. Low to moderately high genetic variations were found among the cultivated clones, while moderately high to high genetic variations were found between accessions of the cultivated clones and the wild species, and also among the wild species. Some cultivated clones were found to have

revealed high level of similarities, and were placed separately within six different clusters.

Three available protocols, those by Gawel and Jarret (1991), Husain (1994) and Mathius and Hutabarat (1997), were used to extract genomic DNA from the leaves of the durian accessions. Mathius and Hutabarat's (1997) protocol was found able to produce the highest quantity of genomic DNA, however, with low quality. Prolongation of incubation period from 30 min to 75 min, addition of TE buffer following cool isopropanol precipitation and CIA (24:1) re-extraction, were found effective to improve Mathius and Hutabarat's (1997) protocol to produce high quality genomic DNA from the durian leaves.

Six pairs of primers i.e. *psb*C, *rpo*B, *rbc*L, *A*, *N* and IGS were used to amplify six specific regions: *psb*C, *rpo*B, *rbc*L, and intergenic spacers of *ndh*C-*trn*V, *atp*B-*rbc*L and IGS-rDNA, respectively. Two primers, *rbc*L and *N*, were found able to consistently amplify the two specific regions, IGS inconsistently amplified the specific and non-specific regions, *A* inconsistently amplified non-specific region, while *rpo*B and *psb*C were found unable to amplify any region at all.

Six restriction enzymes, *Eco* RI, *Bam* HI, *Bsu* RI, *Hind* III, *Pst* I and *Taq* I, were used to digest the PCR products. Four enzymes, *Bsu* RI, *Hind* III, *Pst* I and *Taq* I, were found able to digest the PCR products into smaller fragments, while two enzymes, *Eco* RI and *Bam* HI, were unable to do so.

Generally, enzymes having four-base recognition sites were found able to digest broader range of PCR products than those having six-base recognition sites.

PCR-RFLP on the *ndh*C-*trn*V and *rbc*L regions, using eight restriction enzymes, was conducted to study genetic variation among 11 accessions belonging to 10 species in the genus *Durio*. The polymorphism produced from *ndh*C-*trn*V region showed higher variations than those from *rbc*L region. Based on the results of PCR-RFLP on n*dh*C-*trn*V region, the accessions were grouped into five distinct clusters, while based on results of PCR-RFLP on the *rbc*L region, they were grouped into only three distinct clusters.

PCR-RFLP on the IGS-rDNA and *ndh*C-*trn*V regions in durian DNA from 71 cultivated clones were found to have produced only monomorphic bands, indicating that, no alteration occurred on the pattern of sequences in both regions. Thus, neither genetic distance matrix nor dendrogram tree could be constructed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN MORFOLOGI DAN MOLEKUL KE ATAS GERMPLASMA DURIAN DI MALAYSIA

Oleh

PANCA JAROT SANTOSO

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Pengerusi : Profesor Ghizan Bin Saleh, Ph.D.

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Pengurusan sumber genetik merupakan satu kepentingan bagi pembaikan dan pengeluaran durian yang berterusan. Ianya meliputi penyelenggaraan data-data berkaitan, pencirian dan penilaian ke atas sifat-sifat morfologi yang mudah diperturunkan dan sifat molekul, bagi keperluan kajian taksonomi, pembaikan genetik, jaminan kualiti dan pengurusan. Objektif kajian adalah untuk menentukan tahap variasi di antara germplasma durian di Malaysia mengikut sifat-sifat morfologi dan kaedah PCR-RFLP ke atas DNA kloroplas dan DNA ribosom.

Dalam kajian variasi genetik berasaskan ciri morfologi, sejumlah 65 aksesi durian, yang terdiri dari 60 klon yang ditanam dan lima spesies liar, telah dibandingkan. Sejumlah 12 kelompok yang nyata dikenalpasti memisahkan kesemua aksesi. Variasi genetik yang rendah hingga sederhana tinggi didapati di kalangan klon-klon yang ditanam, manakala variasi genetik yang sederhana tinggi hingga tinggi didapati di antara aksesi-aksesi klon durian

yang ditanam dan spesies liar, dan juga di kalangan spesies liar. Beberapa klon durian yang ditanam didapati menunjukkan tahap persamaan yang tinggi, dan ditempatkan berasingan di dalam enam kelompok yang berbeza.

Tiga kaedah yang sedia ada, iaitu menurut Gawel dan Jarret (1991), Husain (1994), dan Mathius dan Hutabarat (1997), telah digunapakai untuk mengekstrak DNA dari daun aksesi-aksesi durian tersebut. Kaedah Mathius dan Hutabarat (1997) didapati berupaya mengekstrak DNA genom dalam kuantiti terbanyak, walau bagaimanapun, kualitinya adalah rendah. Perlanjutan jangka masa inkubasi dari 30 minit kepada 75 minit, penambahan penimbal TE selepas presipitasi isopropanol sejuk dan ekstraksi semula CIA (24:1), didapati berkesan dalam mengubahsuai kaedah Mathius dan Hutabarat (1997) untuk menghasilkan DNA genom berkualiti tinggi dari daun durian tersebut.

Enam pasang primer, iaitu *psb*C, *rpo*B, *rbc*L, *A*, *N* dan IGS, telah digunakan untuk mengamplifikasi enam kawasan yang khusus: masing-masing *psb*C, *rpo*B, *rbc*L, dan 'intergenic spacer' daripada *ndh*C-*trn*V, *atp*B-*rbc*L dan IGS-rDNA. Dua primer, *rbc*L dan *N,* didapati secara konsisten berupaya mengamplifikasi dua kawasan khusus tersebut, IGS didapati secara tidak konsisten mengamplifikasi kawasan-kawasan khusus dan tidak khusus, *A* secara tidak konsisten mengamplifikasi kawasan tidak khusus, manakala *rpo*B dan *psb*C didapati tidak berupaya mengamplifikasi mana-mana kawasan sama sekali.

Enam enzim penyekat, *Eco* RI, *Bam* HI, *Bsu* RI, *Hind* III, *Pst* I dan *Taq* I, telah digunakan untuk mencerna produk-produk PCR tersebut. Empat enzim, *Bsu* RI, *Hind* III, *Pst* I dan *Taq* I, didapati boleh mencerna produk-produk PCR kepada fragmen-fragmen kecil, manakala dua enzim, *Eco* RI dan *Bam* HI, didapati tidak berupaya melakukanya. Secara amnya, enzim-enzim yang mempunyai tapak pengecam empat-bes didapati mencerna produk-produk PCR pada jangkauan lebih luas berbanding enzim-enzim yang mempunyai tapak pengecam enam-bes.

PCR-RFLP ke atas kawasan-kawasan *ndh*C-*trn*V dan *rbc*L, menggunakan lapan enzim penyekat, telah dilakukan untuk mengkaji variasi genetik antara 11 aksesi yang tergolong kepada 10 spesies di dalam genus *Durio*. Polimorfisme yang dihasilkan daripada kawasan *ndh*C-*trn*V menunjukkan variasi yang lebih tinggi berbanding dengan yang dihasilkan oleh kawasan *rbc*L. Berasaskan kepada keputusan PCR-RFLP ke atas kawasan n*dh*C-*trn*V, aksesi-aksesi tersebut digolongkan ke dalam lima kelompok berasingan, manakala berasaskan keputusan PCR-RFLP ke atas kawasan *rbc*L, ianya digolongkan ke dalam tiga kelompok yang nyata sahaja.

PCR-RFLP ke atas kawasan-kawasan IGS-rDNA dan *ndh*C-*trn*V pada DNA daripada 71 klon durian yang ditanam, didapati menghasilkan hanya jalur-jalur monomorfik, menunjukkan bahawa, tidak ada perubahan yang berlaku ke atas bentuk susunan julat pada kedua-dua kawasan tersebut. Oleh itu, kedua-dua matrik jarak genetik dan pohon dendrogram tidak dapat dibentuk.

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I certify that an Examination Committee met on 20th October 2004 to conduct the final examination of Panca Jarot Santoso on his Master of Science thesis entitled "Morphological and Molecular Assessment of Durian Germplasm in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that this thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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PANCA JAROT SANTOSO

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