UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF DNA BARCODING REGIONS FOR IDENTIFICATION OF MALAYSIAN BANANA CULTIVARS

ABDUL RAHMAN B. ZULKIFLLI

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

ASSESSMENT OF DNA BARCODING REGIONS FOR IDENTIFICATION OF MALAYSIAN BANANA CULTIVARS

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Chair: Associate Professor Siti Khalijah Daud, PhD

Faculty: Science

To date, there is no exclusive barcode for bananas and plantains being documented, hence, this study was undertaken to find out the most suitable barcodes for banana cultivars identification. The aim of this study was to determine the most appropriate DNA regions to be assigned as the DNA barcoding for banana cultivars identification. In this study, genes from chloroplast genome were chosen as the barcode candidates, namely *matK, rpoB, rpoC1, rbcL, trnH-psbA* primers and their combinations. In addition, *ITS2* gene from nuclear genome was also selected.

Six banana cultivars, namely Pisang Jari Buaya, Pisang Raja Udang, Pisang Tanduk, Pisang Rastali, Pisang Nangka and Pisang Nipah were sampled from UPM banana germplasm collection, located in Kompleks Ladang

Bersepadu, Taman Pertanian Universiti, Universiti Putra Malaysia. Four criteria have been used to assess the barcoding properties of the chosen barcode regions, i.e. the ability to be screened and amplified from the whole genome, their genetic divergence characteristic, the ability to form monophyletic clade, and the DNA barcoding gap.

By using the suggested primer pairs, Polymerase Chain Reaction (PCR) technique was employed to screen and amplify the desired regions. The genetic divergence characteristic, inter and intraspecific genetic distances were assessed from the genetic distance matrices based on Kimura 2 Parameter model using MEGA5 software. The monophyletic clade assessment was done following phylogenetic technique. The phylogenetic trees involved were inferred using Neighbour Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) methods. As for the DNA barcoding gap, the data from previous genetic distance matrices were used and the graphs were created using Microsoft Excel 2007 software.

The assessment revealed that the used primers generally performed well in PCR amplification with the average PCR efficiency of 89%. However, *ITS2* showed that it was the easiest marker to work with. On the other hand, *trnH-psbA* has the highest interspecific divergence and the lowest intraspecific divergence (0.0261 and 0.000 respectively). For phylogenetic analysis, *ITS2*

managed to resolve monophyletic clades for all cultivars, which were strongly supported by bootstrap and posterior probability values. Nevertheless, none of the barcode candidates exhibited clear "barcoding gap".

In conclusion, this study revealed that chloroplast genome was not suitable for identification of hybrid plants, such as banana cultivars, because it only can elucidate a single line of genetic inheritance since chloroplast mode of inheritance only either through paternal or maternal line. Nuclear gene, *ITS2*, was shown to be the best candidate for highly hybrid plants such as banana cultivars. Thus, this study suggested that *ITS2* region to be used for DNA barcoding of Malaysian banana cultivars. For future study, the number of cultivars should be increased and Single Nucleotide Polymorphism (SNP) marker can be established based on the barcoding region. By employing High Resolution Melting temperature (HRM) technique, faster, rapid and high throughput identification can be possible.

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

PENILAIAN BARKOD DNA UNTUK PENGENALPASTIAN KULTIVAR PISANG MALAYSIA

Oleh

ABDUL RAHMAN B. ZULKIFLLI

Januari 2013

Pengerusi: Profesor Madya Siti Khalijah Daud, PhD

Fakulti: Sains

Sehingga kini, masih tiada barkod yang ekslusif untuk pisang dan plantain didokumentasikan. Oleh itu, kajian ini dijalankan untuk menentukan barkod yang sesuai bagi pengenalpastian kultivar pisang. Tujuan kajian ini adalah untuk menentukan kawasan barkod yang paling sesuai sebagai pengkodan DNA bagi pengenalpastian kultivar pisang. Dalam kajian ini, gen dari genom kloroplas dipilih sebagai calon barkod, iaitu primer *matK, rpoB, rpoC1, rbcL* dan *trnh-psbA*, serta gabungan antara mereka. Sebagai tambahan, gen *ITS2* dari genom nuklear juga telah dipilih.

Enam kultivar pisang, iaitu Pisang Jari Buaya, Pisang Raja Udang, Pisang Tanduk, Pisang Rastali, Pisang Nangka dan Pisang Nipah telah disampel dari koleksi germplasma pisang UPM yang terletak di Ladang Bersepadu,

Taman Pertanian Universiti, Universiti Putra Malaysia. Empat kriteria telah digunakan untuk menilai sifat-sifat calon barkod yang telah dipilih, iaitu kebolehan untuk disaring dan digandakan dari keseluruhan genom, ciri-ciri pencapahan genetik, kebolehan untuk membentuk klad monofiletik dan jurang barkod DNA.

Dengan menggunakan pasangan primer yang telah dicadangkan, teknik *Polymerase Chain Reaction* (PCR) digunakan untuk menyaring dan menggandakan kawasan yang dikehendaki. Ciri pencapahan genetik, jarak genetik interspesifik dan intraspesifik dinilai daripada matrik jarak genetik yang berdasarkan kepada model Kimura 2 Parameter menggunakan perisian MEGA5. Penilaian klad monofiletik dilakukan dengan menggunakan teknik filogenetik. Pokok filogenetik ini dibina berdasarkan kaedah *Neighbour Joining* (NJ), *Maximum Parsimony* (MP), *Maximum Likelihood* (ML) dan juga *Bayesian Inference* (BI). Untuk analisis jurang barkod DNA pula, data dari matriks jarak genetik yang sama digunakan dan graf jurang barkod dihasilkan dengan menggunakan perisian Microsof Excel 2007.

Penilaian ini menunjukkan pasangan primer yang digunakan pada amnya adalah baik dalam penggandaan PCR dengan purata kecekapan PCR sebanyak 89%. Walau bagaimanapun, *ITS2* menunjukkan yang ia adalah penanda barkod yang mudah untuk dikendalikan. Sebaliknya, *trnH-psbA* mempunyai pencapahan interspesifik paling tinggi (0.0261) dan pencapahan

intraspesifik yang paling rendah (0.000). Untuk analisis filogenetik pula, *ITS2* mampu untuk merungkai klad monofiletik bagi semua kultivar pisang yang terlibat yang mana ia disokong kuat oleh nilai *bootstrap* dan nilai kebarangkalian posterior. Namun begitu, tidak ada satu pun calon barkod yang berjaya mempamerkan jurang barkod yang jelas.

Sebagai kesimpulan, kajian ini menunjukkan bahawa genom kloroplas tidak sesuai bagi pengenalpastian tumbuhan hibrid, seperti kultivar pisang, kerana ia hanya mampu menjelaskan satu arah pewarisan genetik sahaja. Ini adalah kerana mod pewarisan kloroplas diwariskan hanya melalui bapa atau ibu sahaja. Gen nukleus, *ITS2,* didapati sangat baik digunakan bagi pengenalpastian tumbuhan yang sangat hibrid seperti kultivar pisang. Oleh itu, kajian ini mencadangkan supaya kawasan *ITS2* digunakan sebagai penanda barkod DNA untuk kultivar pisang Malaysia. Untuk kajian akan datang pula, bilangan kultivar pisang perlu ditambah dan penanda *Single Nucleotide Polymorphism* (SNP) pula boleh digunakan berdasarkan kawasan pengkodan ini. Dengan menggunakan teknik *High Resolution Melting temperature* (HRM) pula, pengenalpastian yang cepat, pantas dan secara besar-besaran dapat dilakukan.

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Thank you.

ABDUL RAHMAN ZULKIFLLI

JANUARY 2013

I certify that an Examination Committee has met on 11th January 2013 to conduct the final examination of Abdul Rahman b. Zulkiflli on his Master of Science thesis entitled "Assessment of DNA barcoding regions for identification of Malaysian banana cultivars" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Masters of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee were as follows:

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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 at any other institution.

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ABDUL RAHMAN B. ZULKIFLLI

Date:

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