UNIVERSITI PUTRA MALAYSIA

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MASTER OF SCIENCE
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By

KHOO SIEW PING

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

June 2012

Dedicated to my loving parents, Kang Hian and Yoke Ying and lovely brothers, Chee Kwong, and sisters, Siew Chian, Siew Yong and Siew Fen, and my beloved partner, Khong Hau

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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Chair: Cheah Yoke Kqueen, PhD

Faculty: Medicine and Health Sciences

The employment of genetically modified organisms as food and feed products in the market is increasing dramatically from year to year. The estimated global area planted with genetically modified crops has reached 148 million heactares involving 29 countries in year 2010. Important genetically modified traits include herbicide tolerance traits encoded by the *5'-enolpyruvylshikimate-3-phosphate synthase* (*Cp4 epsps*) gene derived from *Agrobacterium sp.* and insect resistance traits encoded by the *cry* gene derived from *Bacillus thuringiensis.* Worldwide commercialization of food and feed products containing the genetically modified material has raised concern from the public regarding food safety issues as well as destruction of biodiversity. Thus, many countries have established labeling legislation for genetically modified food and feed products based on a certain threshold level of their genetically modified content. The stakeholders of food and feed product companies are required to comply with the legislation to import or export their products globally.

To date, several different screening and quantification assays have been developed depending on either protein or DNA as target molecules. The purpose of this study was to detect the presence of herbicide resistance Roundup ready soybean (RRS), insect resistance BT maize event 176 and insect resistance BT maize event CBH 351 (tradename StarLink corn) in food and feed products collected from the Malaysian market, by utilizing the polymerase chain reaction (PCR) assay. This study also aimed to develop a quantitative system to determine the percentage of genetically modified organism content in samples by using the SYBR Green I based real-time polymerase chain reaction assay. In this system, recombinant plasmid DNA is generated and used as a calibrator to establish calibration curves.

The results from the PCR assay show that 108 out of 115 (94%) soybean containing samples were positive for the soybean *lectin* gene, and 45 out of 108 (42%) samples were positive for the *Cp4 epsps* gene specific to RRS including animal feeds (31), processed food (13) and raw soybean (1). Quantitative results showed 34 out of 45 (76%) samples certainly more than 0.9% of the Roundup ready soybean.

PCR assay showed 80 out of 104 (77%) maize containing samples to be positive for the maize *invertase* gene; and quantitative results showed 6 samples positive for BT maize event CBH 351 and 1 sample positive for BT maize event 176. The percentage of StarLink corn certainly positive GMO samples ranged from 0.09% to 2.53%. However, the one sample positive for BT maize event 176 was shown to certain 16.90% of BT maize event 176.

In conclusion, the developed qualitative and quantitative system to determine the presence of genetically modified organism in various kinds of samples in the present study could contribute towards the better post-market surveillance and facilitate the implementation of the labeling process.

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

PENGESANAN ORGANISMA TERUBAH SUAI SECARA GENETIK BAGI SIFAT RINTANGAN TERHADAP SERANGGA AND HERBISID DENGAN KAEADAH KUALITATIF DAN KAEDAH KUANTITATIF BERDASARKAN PLASMID

By

KHOO SIEW PING

Jun 2012

Pengerusi: Cheah Yoke Kqueen, PhD

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Kewujudan pengunaan organisma yang dihasilkan oleh teknik kejuruteraan genetik sebagai makanan dan produk makanan haiwan di pasaran telah meningkat drastik dari tahun ke tahun. Tanaman yang berasal daripaada hasil kejuruteraan genetik telah ditanami di seluruh dunia dan dijangka telah mencapai 148 juta heactare dan melibatkan 29 negara pada tahun 2010. Sifat hasil kejuruteraan genetik yang utama termasuk sifat rintangan terhadap herbisid yang dikodkan oleh gen *5'-enolpyruvylshikimate-3-phosphate synthase* (*Cp4 epsps*) berasal dari *Agrobacterium sp* dan rintangan terhadap serangga yang dikodkan oleh gen *cry* berasal dari *Bacillus thuringiensis*. Komersialisasi makanan dan produk makanan haiwan di pasaran telah manarik perhatian awam tentang isu keselamatan makanan dan juga perlupusan keanekaragaman hayati. Oleh demikian, banyak negara telah melaksanakan undang-undang pelabelan untuk makanan dan produk makanan haiwan yang mengandungi tahap isi hasil organisma kejuruteraan

genetik yang tertentu. Pemegang amanah harta untuk syarikat tertentu yang terlibat dalam rangkaan mengimport dan mengeksport produk secara global perlu mematuhi undang undang yang ditetapkan. Dengan demikian, beberapa jenis analisasi kualitatif and kuantitatif terhadap hasil organisma kejuruteraan genetik yang bergantung kepada protein atau DNA telah dikembangkan.

Tujuan kajian ini adalah untuk mengesan kewujudan hasil organisma kejuruteraan genetik seperti Roundup ready soya yang mengandungi sifat rintangan terhadap herbisida, BT176 and CBH 351 jagung yang mengandungi sifat rintagan terhadap serangga dalam sampel yang dikumpulkan dari pasaran di Malaysia dengan menggunakan teknik PCR. Selain itu, sistem kuantitatif untuk menentukan peratusan kewujudan organisma kejuruteraan genetik dalam sampel dengan menggunakan teknik SYBR Green I berdasarkan Real-time PCR juga dijalankan dalam kajian ini. Dalam system kuantitatif ini, rekombinan plasmid DNA telah dibentukan dan juga digunakan sebagai kalibrator untuk menghasilkan graf lengkung piawai.

Daripada 115 sampel yang mengandungi soya, 94% dari jumlah keseluruhan telah dikesan positif terhadap gen *lektin* soya. Selain itu, di antara 45 dari 108 sampel adalah ditunjukan positif kepada gen *Cp4 epsps* yang khusus terhadap Roundup ready soya, termasuk makanan ternakan haiwan (31), makanan manusia (13) dan biji soya (1). Namun, keputusan kuantitatif menunjukkan 75.6% (34/45) sampel yang dilaporkan positif mengandungi lebih daripada 0.9% Roundup ready soya. Selain itu, 80 daripada 104 sampel yang mengandungi jagung adalah ditunjukkan positif terhadap gen jagung

invertase. Dalam kajian ini, 6 sampel dikesan positif untuk CBH 351 jagung serta 1 sampel positif untuk BT176 jagung. Peratusan daripada jagung CBH 351 yang terkandung dalam sampel positif adalah di antara 0.09% dan 2.53%. Namun demikian, satu sampel jagung yang positif terhadap BT176 jagung telah dilaporkan mangandungi 16.90% jagung BT176.

Kesimpulannya, sistem kualitatif dan kuantitatif yang telah dihasilkan untuk menentukan kewujudan organisma kejuruteraan genetik dalam pelbagai jenis sampel dalam kajian ini boleh membantu pengawasan makanan yang mengandungi organima kejuruteraan genetik dalam pasaran dengan lebih baik dan juga memudahkan pelaksanaan proses pelabelan.

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I certify that a Thesis Examination Committee has met on 15th June 2012 to conduct the final examination of Khoo Siew Ping on her thesis entitled "Genetically Modified Organism Detection of Insect and Herbicide Resistance Traits by Qualitative and Quantitative Plasmid-based Methods" in accordance with the Universities and University College 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

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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 Master 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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KHOO SIEW PING

Date: 15 June 2012

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