



UNIVERSITI PUTRA MALAYSIA

**GENE CLONING, PROTEIN CHARACTERIZATION AND STRUCTURAL
PREDICTION OF *Amaranthus tricolor* TRANSKETOLASE**

ALLGENDRAN A/L RAJEENDRAN

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By

ALLGENDRAN A/L RAJEENDRAN

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

MAY 2013

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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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By

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MAY 2013

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This study focused on gene cloning, protein characterization and structural prediction of Transketolase enzyme from *Amaranthus tricolor*. Oxidative Pentose Phosphate Pathway (OPPP) oxidative pentose phosphate pathway (OPPP) fulfills an essential role in metabolite intermediate of carbohydrate metabolism via the oxidation of glucose-6-phosphate in prokaryotic and eukaryotic cells. The non-oxidative branch of this pathway provides erythrose 4-phosphate for the shikimate pathway, the products of which are used for the synthesis of aromatic amino acids, flavonoids, and lignin, and ribose 5-phosphate for nucleic acid synthesis. Transketolase play important role in photosynthesis and glycolysis. Although the contribution of transketolase in plant system is important, study of this enzyme is still limited. There were only seven plants that *TKT* genes had been isolated so far, thus this leads to my first objective of my study is to isolate *TKT* gene from *A. tricolor* to compare its identity with other organisms. Unlike bacteria, fungal and all other animals, the pentose phosphate pathway is

complete in the cytosol of these living system and all enzymes of this pathway localized in the cytosol. However, in plant system, the first phase of pentose phosphate pathway is complete in the cytosol of plant but sub-localization for non-oxidative pentose phosphate pathway is still remain unclear. The identification gene of *TKT* was done by RT-PCR with gene specific primer, followed by cloning, sequencing and gene expression. The present study had isolated 1963bp transketolase from *A. tricolor*. Similarity studies using ClustalW2 and phylogenetic studied found that *A. tricolor TKT* and *Spinach sp.* transketolase has 85% identity. This may be due to classification system both plants are in the same family, Amarathaceae. Analysis by Target P 1.1 and Chloro P revealed that the *A. tricolor* is localized in the chloroplast. The complete DNA sequence of the *A. tricolor TKT* gene was showed an open reading frame of 1943 nucleotides that encodes for 646 amino acid residues. *A. tricolor TKT* was expressed in *E.coli* BL21, using pET 32(b+) as a vector. The purified recombinant *A. tricolor TKT* protein size was determined by SDS-PAGE that give 70kDa. In addition, the 3D protein structure of *A.tricolor* transketolase was analysed by using PS² and secondary its structure was predicted by Phyre2. As conclusion, *A. tricolor* transketolase was successfully isolated and subcellular-localization was found chloroplast in plant system. This led to conclusion that the OPPP is incomplete in the cytosol of *A. tricolor*. This study shows that *A.tricolor* transketolase has major functional role in the plastidic through the OPPP and could be an important required step for the production of secondary metabolites in plants.

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

**GEN KLON, PROTEIN PENCIRI-CIRIKAN DAN KAJIAN STRUKTUR BAGI
Amaranthus tricolor TRANSKETOLASE**

Oleh

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Kajian ini memfokuskan kepada pemencilan, penciri-cirikan dan kajian struktur bagi *Amaranthus tricolor* Transketolase. Tapak jalan Pentosa Fosfat (OPPP) terdiri daripada dua fasa, iaitu, oksidatif dan bukan-oksidatif. Fungsi OPPP terutamanya untuk menjana NADPH dan prekursor untuk proses biosintetik pelbagai kompaun sebagai blok binaan asas kehidupan. Salah satunya adalah transketolase yang memainkan peranan penting dalam fasa bukan-oksidatif di mana menghasilkan beberapa pengantara gula fosfat. Erythrosa-4-fosfat akan memasuki tapak jalan shikimate dimana tapak jalan ini merupakan tapak jalan yang penting dalam penghasilan metabolit sekunder dalam sistem tumbuhan seperti asid amino, lignin dan flavonoid. Transketolase also memainkan peranan penting dalam proses fotosintesis dan glikolisis. Oleh sebab itu peranan transketolase dalam sistem tumbuhan adalah penting, tetapi kajian mengenai enzim ini dalam tumbuhan adalah terlalu terhad. Sehingga kini, pemencilan Transketolase gen telah dilakukan dari tujuh spesis sahaja. Dibandingkan dengan

bacteria, kulat dan haiwan dalam proses OPPP adalah lengkap. Tetapi, sistem tumbuhan, fasa pertama adalah lengkap dalam sitosol, tetapi fasa kedua masih dalam kajian. Kajian ini telah berjaya mengasingkan jujukan nukleotida transketolase dengan menguna Clustal W2 dan pilogenetik menunjukkan bahawa *A. tricolor* TKT and *Spinach* sp. transketolase adalah persamaan dengan 85% dan berbanding dengan tumbuhan lain. Penempatan subsellular untuk *A.tricolor* transketolase telah meramal dengan mengguna Target P 1.1 and Chloro P menunjukkan bahawa dalam kloroplast. *A. tricolor* TKT gene yang dikaji telah ditunjukkan untuk mengandungi rangka bacaan dengan sebanyak 1943bp nukleotida dengan mengekod untuk 646 residu asid amino. Recombinan *A. tricolor* TKT telah disertakan dalam *E.coli* BL21, menggunakan pET 32 (a+) sebagai vector. Rekombinan saiz protein *A. tricolor* TKT telah ditentukan dengan mengguna SDS-PAGE dengan 70 kDa saiz protein. Tambahan lagi, 3D protein struktur bagi *A. Tricolor* transketolase telah menjangka dengan menguna PS² dan struktur sekunder telah meramal dengan menguna Phyre 2. Sebagai kesimpulan, jujukan nukleotida TKT bagi *A. tricolor* telah berjaya dipencilkan. Oleh itu, kajian ini mendapati bahawa OPPP adalah tidak lengkap dalam sitosol tumbuhan. Kajian ini, mennunjukkan bahawa *A. tricolor* transketolase adalah gen yang sangat penting dalam laluan OPPP dan pengeluaran kompaun sekunder dalam tumbuhan.

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I certify that a Thesis Examination Committee has met on 7 May 2013 to conduct the final examination of Allegendran A/L Rajeendran on his thesis entitled "Molecular isolation, characterization and structural prediction of *Amaranthus Tricolor* Transketolase" 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.

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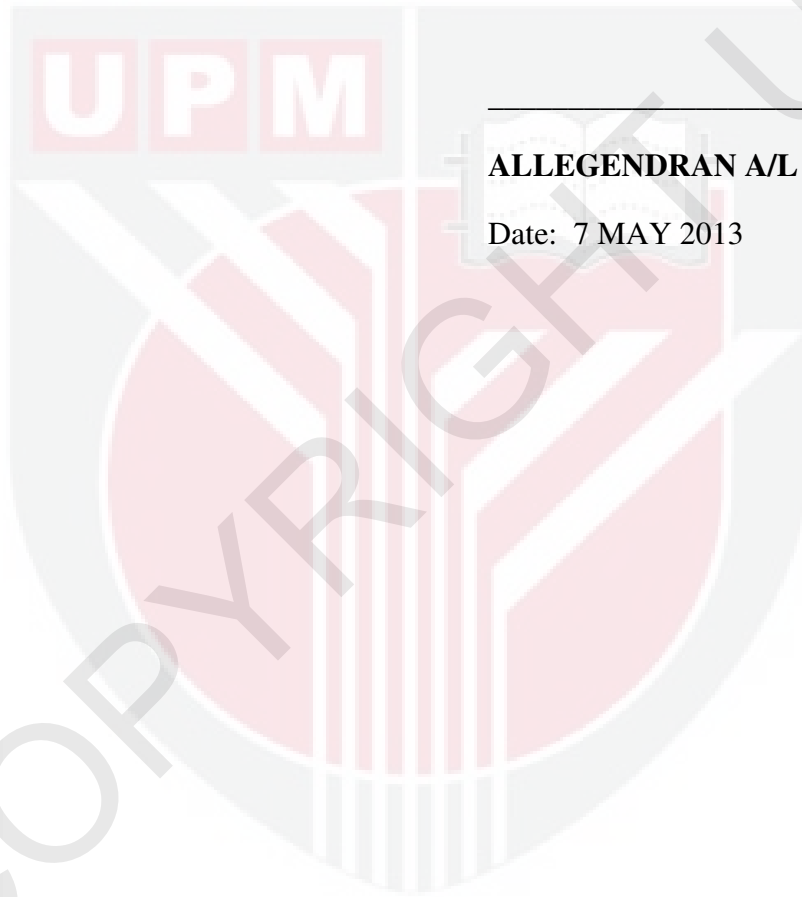
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DECLARATION

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 University Putra Malaysia or other institutions.



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