IDENTIFICATION AND ANALYSES OF GENES DIFFERENTIALLY EXPRESSED DURING GRAIN FILLING IN INDICA RICE (*Oryza sativa* L.) VARIETY MR 84

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IDENTIFICATION AND ANALYSES OF GENES DIFFERENTIALLY EXPRESSED DURING GRAIN FILLING IN INDICA RICE (*Oryza sativa* L.) VARIETY MR 84

By

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Chairman : Associate Professor Suhaimi bin Napis, PhD

Faculty : Biotechnology and Biomolecular Sciences

Rice is one of the major crops in Malaysia. It has been a staple food of the Malaysian community for many decades. Continued growth of Malaysian population put additional pressure on local rice production to meet the demand of the consumption. Rice grain filling is a critical factor that directly affects the local rice production. This preliminary study was carried out to identify genes that are putatively involved in the grain filling of rice by using cDNA subtraction. Four subtracted cDNA libraries were constructed from three different pools of RNA (samples A, B and C) during the reproductive and ripening phase of rice. A total of 2366 clones were obtained from these four subtracted cDNA libraries and 384 clones were sequenced. In sample A (panicle initiation), most of the sequenced cDNA were putatively involved in cell wall structure and metabolism (38 %) e.g. pollen allergen (subtraction 1). The number of putative genes involved in metabolism (9 %) and storage protein (3 %) was low in this library (subtraction 1). The genes that were differentially expressed in heading to milk stage (sample B) were mainly

involved in metabolism (28 %, subtraction 3). Most of the genes that were differentially expressed in sample C (milk stage to maturation) were putative storage proteins (61 %) e.g. glutelins and prolamins, and the genes that were putatively involved in metabolism (10 %) decreased compared to subtraction 3. This reflected that metabolic activities in panicles significantly reduced and most of the activities in grain were involved in reserving food towards the end of the grain filling. To understand the grain filling process during rice growth stages, ADP-glucose pyrophosphorylase which is the rate-limiting enzyme in starch biosynthesis was studied. The cDNA for the 3' region of ADP-glucose pyrophosphorylase small subunit (Adp3'-2) (which showed 97 % identity to ADP-glucose pyrophosphorylase small subunit from japonica cultivar) was isolated. It was preferentially expressed in milk and dough stage of grain development. Southern analysis showed that Adp3'-2 may be a single copy gene in the rice genome. In addition, bZIP protein which has a regulatory role in several plant developmental processes including seed storage has also been cloned. The cDNA for the 3' region of bZIP protein was also isolated. No signal was detected in Northern analysis attributed to its low expression in panicle, and the Southern analysis also suggested that 3' Reb-2 may be a single copy gene in the rice genome.

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

IDENTIFIKASI DAN ANALISIS GEN-GEN YANG TERLIBAT DALAM PENGISIAN BIJIAN PADI INDICA (*Oryza sativa* L.) VARIATI MR84

Oleh

WONG YICK CHING

April 2006

Pengerusi : Profesor Madya Suhaimi bin Napis, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Padi merupakan salah satu tanaman utama di Malaysia. Ia merupakan makanan utama kepada komuniti Malaysia. Pertumbuhan populasi penduduk Malaysia yang berterusan telah memberi tekanan kepada pengeluar padi tempatan untuk memenuhi keperluan permintaan penduduk negara ini. Pengisian bijian padi merupakan satu faktor kritikal yang mempengaruhi pengeluaran padi tempatan secara langsung. Pangajian ini dijalankan untuk mengidentifikasikan calon gen yang terlibat dalam pengisian bijian padi dengan menggunakan kaedah cDNA subtraktif. Empat perpustakaan cDNA subtraktif telah dibina daripada tiga kumpulan RNA yang berlainan (sampel A, B dan C) dalam fasa reproduktif dan kemasakan padi. Sebanyak 2366 klon telah didapati daripada empat perpustakaan cDNA subtraktif dan 384 klon daripadanya telah dijujukkan. Dalam sampel A (peringkat kemunculan panikel ke peringkat 'heading'), kebanyakkan klon cDNA yang terpencil berkemungkinan terlibat dalam struktur dinding sel dan metabolisma (38 %) contohnya 'allergen' debunga (Subtraktif 1). Gen yang berkemungkinan terlibat dalam metabolisma (9 %) dan protein penyimpanan (3 %) adalah rendah dalam perpustakaan ini (Subtraktif 1). Kebanyakkan gen yang terzahir dalam peringkat 'heading' ke peringkat 'milk' (sampel B) terlibat dalam metabolisma (28 %) (Subtraktif 3), manakala kebanyakkan gen yang terzahir dalam sampel C (peringkat 'milk' ke peringkat masak) berkemungkinan terlibat dalam penyimpanan protein (61 %) seperti glutelin

dan prolamin. Calon-calon gen yang terlibat dalam metabolisma (10 %) telah menurun berbanding dengan Subtraktif 3. Ini menunjukkan aktiviti metabolisma dalam panikel telah mengurang dan kebanyakkan aktiviti bijian di peringkat akhir pengisian bijian terlibat dalam penyimpanan makanan. Untuk memahami proses pengisian bijian semasa peringkat pertumbuhan padi, ADP-glukosa pirofosforilase yang merupakan enzim penghad kadar dalam biosintesis kanji telah dikaji. Klon cDNA bahagian 3' subunit kecil ADP-glukosa pirofosforilase (Adp3'-2) yang menunjukkan 97 % identiti dengan subunit kecil ADP-glucose pyrophoaphorylase daripada kultivar japonika telah dipencilkan. Ia terzahir pada peringkat 'milk' dan 'dough' dan hanya di panikel. Analisa 'Southern' menunjukkan bahawa Adp3'-2 berkemungkinan adalah gen yang mempunyai satu salinan dalam padi. Selain itu, protein bZIP yang berperanan sebagai pengawalatur beberapa proses perkembangan tumbuhan termasuk penyimpanan benih telah turut diklonkan. Klon cDNA bahagian 3' bagi protein bZIP (3' Reb-2) juga telah dipencilkan. Analisa 'Northern' tidak dapat mengesan penzahiran gen ini disebabkan tahap penzahirannya yang rendah dalam panicle, and analisa 'Southern' juga mencadangkan bahawa 3' Reb-2 berkemungkinan wujud sebagai gen satu salinan dalam genom padi.

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I certify that an Examination Committee has met on 7th April 2006 to conduct the final examination of Wong Yick Ching on his Master of Science thesis entitled “Identification and Analyses of Genes Differentially Expressed During Grain Filling in Indica Rice (*Oryza sativa* L.) Variety MR 84 “ in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee

recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis 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 are as follows:

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DECLARATION

I hereby declare that the 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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WONG YICK CHING

Date:

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