UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF OIL PALM (Elaeis guineensis Jacq) RNAi CONSTRUCTS AND TRANSFORMATION OF cDNA CANDIDATES INTO RICE (Oryza sativa L)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment 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.

DEVELOPMENT OF OIL PALM (*Elaeis guineensis* Jacq) RNAi CONSTRUCTS AND TRANSFORMATION OF cDNA CANDIDATES INTO RICE (*Oryza sativa* L)

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August 2012

Chair : Associate Professor Ho Chai Ling, PhD

Faculty : Biotechnology and Biomolecular Sciences

The current rate of oil palm embryogenesis in the industry ranges from 3 % to 6 %, and is an acknowledged obstacle in scaling up tissue culture production. Isolation of cDNA candidates that may have potential involvement in the oil palm somatic embryogenesis has been carried out in previous studies. In this study, four oil palm cDNA candidates (EgPER1, EgHOX1, OPSC10 and EgPK1) were chosen for functional analysis studies. Construction of RNAi vectors and rice transformation using the overexpression vectors were performed. The PCR products were amplified from full length cDNA candidates that were previously cloned into the intermediate vector, pDONR221 and cloned into pANDA vector with LR clonase enzyme. The positive clones obtained from the LR reaction were screened with PCR in the sense and antisense direction and verified by sequencing. All four cDNA candidates which

have been cloned into the overexpression vector, pMDC32 driven by a double cauliflower mosaic virus (CAMV) were transformed into Taipei 309 rice. The calli transformed with pMDC32/OPSC10 failed to regenerate on normal regeneration medium. The calli had slow growth rate and was stunted, leading to phenotypic aberrations. Modifications of the regeneration medium by completely removing sucrose and adding high cobalt concentration (100 μM) promoted regeneration of the stunted calli. Although several calli were obtained from the transformation, only one plantlet survived while others displayed albinism and failed to revert to normal growth on the modified regeneration medium. The plantlet had a drastic increase in height in 14 days once transferred onto the modified regeneration medium. However, it did not survive outside the tissue culture environment. The putative transformants obtained from the subsequent transformation were screened with PCR using four different sets of primers (nosT, hygromycin, 35 S and gene specific forward). Only one line transformed with pMDC32/EgPK1 showed consistent results with all four primers. Southern blot analysis of PCR products generated using gene specific primers confirmed that the EgPK1 was successfully integrated into the rice genome. This transformant was phenotypically normal. The results obtained were preliminary but will provide guidance for further analysis of EgPK1 and OPSC10 to verify their functions in oil palm somatic embryogenesis.

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

PENGHASILAN KONSTRUK RNAi KELAPA SAWIT (*Elaeis guineensis* Jacq) DAN TRANSFORMASI CALON cDNA KE DALAM PADI (*Oryza sativa* L)

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Kadar semasa embriogenesis industri kelapa sawit masih di antara 3 % hingga 6 %, dan merupakan faktor penghalang utama untuk meningkatkan penghasilan kultur tisu pada skala besar. Pemencilan calon cDNA yang berpotensi dalam penglibatan proses embriogenesis somatik kelapa sawit telah dijalankan dalam penyelidikan yang terdahulu. Dalam kajian ini, empat calon cDNA (EgPER1, EgHOX1, OPSC10 dan EgPK1) telah dipilih untuk kajian kefungsian. Penghasilan konstruk RNAi dan transformasi padi menggunakan konstruk pengekspresan berlebihan telah dijalankan. Produk PCR yang telah diamplifikasi daripada jujukan lengkap calon cDNA yang telah diklonkan ke dalam vektor perantara, pDONR221 dan diklonkan ke dalam vektor pANDA dengan enzim LR Clonase. Klon positif yang diperolehi daripada reaksi LR Clonase disaring dengan PCR pada arah ke depan dan ke belakang dan disahkan melalui penganalisaan jujukan. Kesemua empat calon cDNA yang telah

diklonkan ke dalam vektor pengekspresan berlebihan, pMDC32 yang mempunyai promoter berganda Cauliflower Mosaic Virus (CAMV) telah ditransformasikan ke dalam padi Taipei 309. Kalus yang telah ditransformasi dengan pMDC32/OPSC10 gagal menjalani regenerasi pada medium regenerasi biasa. Kalus melalui pertumbuhan yang perlahan atau terbantut yang menyebabkan keabnormalan fenotipik. Pengubahsuaian ke atas medium regenerasi dengan mengeluarkan sumber karbon dan menambahkan ion kobalt berkepekatan tinggi (100 μM) menggalakkan regenerasi kalus yang terbantut. Walaupun beberapa kalus telah diperolehi daripada transformasi, hanya satu kalus terus hidup manakala kalus lain menunjukkan ciri albino dan gagal melalui pertumbuhan normal di atas medium regenerasi yang telah terubahsuai. Anak padi mempunyai peningkatan ketinggian yang drastik dalam 14 hari setelah dipindahkan ke medium regenerasi yang telah diubahsuai. Walaupun begitu, anak padi tersebut tidak dapat hidup di luar keadaan kultur tisu. Transforman putatif diperolehi daripada transformasi berikutnya telah disaring dengan PCR menggunakan empat jenis pasangan pencetus PCR (nosT, higromisin, 35 S dan spesifik gen ke depan). Hanya satu transforman putatif yang ditransformasi dengan pMDC32/EgPK1 menunjukkan keputusan yang konsisten dengan kesemua empat pasangan pencetus PCR. Analisis Southern Blot menggunakan produk PCR yang dihasilkan menggunakan pencetus PCR spesifik gen mengesahkan bahawa EgPK1 telah berjaya diintegrasi ke dalam genom padi. Transforman putatif ini mempunyai fenotipik normal. Keputusan yang diperolehi masih pada peringkat awal dan boleh dijadikan panduan untuk analisis seterusnya bagi EgPK1 dan OPSC10 untuk

mengesahkan fungsi mereka dalam embriogenesis somatik kelapa sawit.

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This thesis was submitted to the Senate of Universiti Putra Malaysia 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.

KALAI VANI A/P MANIAM

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CHAPTER 1

INTRODUCTION

Somatic embryogenesis (SE) involves developmental restructuring of somatic cells towards the embryogenic pathway, and forms the basis of cellular totipotency in higher plants (Chugh and Khurana, 2002). Research on somatic embryogenesis is often focused on two main factors: callogenesis and the embryogenesis rate. Embryogenesis rate is extremely important as it determines the number of plantlets that will be regenerated from the callus of a particular plant. However, after two decades of research, the callogenesis rate for the oil palm is 19 % with an average of 6 % embryogenesis rate in the industry (Wooi, 1993). This reported figure has not changed much throughout the years although variations may exist between the laboratories The oil palm tissue culture process has remained fraught with difficulties (Chan et al. 2010).

The oil palm industry was estimated to face a loss of approximately RM 80,000 per year if oil palm materials were cloned without any prior screening for their embryogenic potential or tissue culturability (Abdullah and Ooi, 2007). The identification of genes that are unique in the embryogenic tissues of oil palm clones can facilitate the screening process to distinguish embryogenic from non embryogenic clones at the tissue culture stage itself. Thus, the identification of genes that may have potential involvement in the various stages of somatic embryogenesis

has been given priority. Previous studies were focused on the isolation of cDNA candidates from the oil palm suspension cultures and their preliminary expression in embryogenic calli was tested (Ong, 2000; See, 2002; Ooi, 2003). Four of the cDNA candidates (EgPER1, EgHOX1, OPSC10 and EgPK1) were chosen for functional analyses in this study.

Functional analysis of these cDNAs was conducted by developing RNAi constructs and transforming the overexpression vectors into rice. RNA silencing is a widely applied method due to its ability in the control of gene expression by suppression (Horiguchi, 2004). In this study, the cDNAs were cloned into the expression vector, pANDA in the sense and antisense direction with an intron as spacer. Inclusion of an intron in silencing constructs was found to have consistently enhancing effect in plants (Wesley et al. 2001).

Overexpression was conducted by placing the open reading frame (ORF) of a gene under the transcriptional control of any constitutively expressed promoter. The vector, pMDC32 with a double constitutive Cauliflower Mosaic Virus (CAMV) promoter was used to conduct overexpression studies of the oil palm cDNAs in this study. The production of a protein in abundance in the plants may provide a phenotype that may help to elucidate its functions (Curtis and Grossniklaus, 2003). However, overexpression does not always result in phenotypic aberrations as plants may appear normal due to internal compensation mechanism. Overexpression may

also lead to failure in regeneration or lethality in plants due to severe impairment in physiological functions that prevent survival.

Transformation was conducted with rice as both rice and oil palm belong to the monocotyledon group. Rice has the advantage of being a model monocotyledon plant which can be manipulated to understand other agronomically important grass genomes (Ware et al. 2002). Rice transformation with *Agrobacterium tumefaciens* has been established for the japonica rice (Hiei et al. 1997) and provides a suitable platform for functional analysis.

In this study, efforts have been taken to conduct functional analysis on the oil palm cDNA candidates to verify their potential involvement in the somatic embryogenesis of oil palm through the overexpression and development of RNAi vectors. This study has three main objectives:

1. To transform the overexpression constructs of OPSC10 and EgPK1 into rice.

2. To analyze the transgenic rice with overexpression of EgPK1 and OPSC10.

3. To construct RNAi vectors for the oil palm cDNA candidates (EgPER1, EgHOX1, EgPK1 and OPSC10).

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