UNIVERSITI PUTRA MALAYSIA

FUNCTIONAL ANALYSES OF PUTATIVE BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 AND PUTATIVE ETHYLENE TRANSCRIPTIONAL COACTIVATOR FROM MALAYSIAN INDICA RICE VARIETY (Oryza sativa L.) MR219

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By

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FUNCTIONAL ANALYSES OF PUTATIVE BRASSINOSTEROID INSENSITIVE 1- ASSOCIATED RECEPTOR KINASE 1 AND PUTATIVE ETHYLENE TRANSCRIPTIONAL COACTIVATOR FROM MALAYSIA INDICA RICE VARIETY (*Oryza sativa* L.) MR219

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Chairman: Associate Professor Ho Chai Ling, PhD

Faculty: Biotechnology and Biomolecular Sciences

Phytohormones integrate metabolic and developmental signals in plants. Active BRI1-Associated Kinase I (BAK1) is required to interact with Brassinosteroid Insensitive 1 for brassinosteroid perception and signal transduction that regulate plant growth and development while ethylene transcriptional co-activator (ETC) acts on ethylene-responsive transcription factors, which positively control the expression of ethylene-responsive plant genes to elicit a response appropriate to the stimulus. The aim of this study is to annotate the functions of two putative phytohormone-related transcripts, *OsI-BAK1* and *OsI-ETC* from a Malaysian rice variety, MR219. Functional annotation of phytohormone-related genes, which are closely related to yield, may offer new solution to increase rice yield in the country. In the present study, the function of *OsI-BAK1* and *OsI-ETC* were investigated using a transgenic approach through gene overexpression and RNAi silencing. In this study, one overexpression vector, pMDC32-BAK1 and two RNAi vectors, pANDA-BAK1 and pANDA-ETC were constructed. These vectors, together with an existing overexpression vector pMDC32-ETC were transformed into rice MR219 through *Agrobacterium*-mediated transformation, respectively. After plant transformation, the phenotypic and molecular changes on the transgenic rice plants were analyzed. The transgenic rice plants overexpressing *OsI-BAK1* had corrugated and twisted leaves at the early stage of growth. Histological analysis of these leaves showed some differences in their cell arrangement in the vascular tissue compared to that of the untransformed plants. The bulliform cells which were enlarged and plugged deep into leaf epidermis in these leaves could have contributed to the abnormal morphology of the leaf blades. Silencing of *OsI-BAK1* in rice plants produced about two folds higher number of undeveloped green and unfilled grains compared to untransformed plants. Histological analyses were conducted on the rice hull, culms and leaves in *Os*I-BAK1RNAi plants. The longitudinal section of rice hull from *Os*I-BAK1RNAi plants showed that the embryo was either absent or retarded in its development. Taken together, *OsI-BAK1* gene may play an important role in the developmental processes of seeds and leaf cell arrangement. Embryogenic calli

transformed with the overexpression vector harboring *OsI-ETC* could not regenerate. The constitutive expression of *OsI-ETC* in rice might increase the production of ethylene and inhibit shoot regeneration. The transgenic plant containing a construct designed for RNAi silencing of *OsI-ETC* showed no obvious morphological difference compared to that of untransformed plant. As it is possible that this construct did not actually function and the plant may not have been RNA silenced to give obvious phenotypic changes. Functional analysis of *OsI-BAK1* and *OsI-ETC* in this study has shed light on their biological functions in rice.

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

ANALISIS FUNGSI PUTATIF "BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1" DAN PUTATIF "ETHYLENE TRANSCRIPTIONAL COACTIVATOR" DARIPADA PADI MALAYSIA INDICA VARIETI (*Oryza sativa* L.) MR219

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Fitohormon mengintegrasikan isyarat metabolik dan perkembangan dalam tumbuhan. Brassinosteroid Sensitif 1 (BRI1) yang aktif diperlukan untuk berinteraksi dengan BRI1-Associated kinase I (BAK1) untuk persepsi brassinosteroid dan transduksi isyarat yang mengawalatur pertumbuhan dan perkembangan tumbuhan sementara ko-aktivator transkripsi etilena (ETC) bertindak ke atas faktor transkripsi yang respon terhadap etilena, dan mengawal ekspresi gen secara positif terhadap etilena untuk mendapatkan tindak balas yang sesuai dengan rangsangan. Tujuan kajian ini adalah untuk menganotasikan fungsi dua transkrip yang berkaitan dengan fitohormon iaitu, *OsI-BAK1* dan *OsI-ETC* daripada padi Malaysia indica varieti MR219. Anotasi fungsi gen yang berkaitan dengan fitohormon yang mempengaruhi hasil padi menawarkan penyelesaian baru untuk meningkatkan penghasilan padi di negara ini. Dalam kajian ini, penyelidikan atas fungsi *OsI-BAK1* dan *OsI-ETC* telah dijalankan menggunakan pendekatan transgenik melalui pengzahiran gene berlebihan dan RNAi perendam gen. Dalam kajian ini, satu vektor pengzahiran gen berlebihan, pMDC32-BAK1 dan dua vektor RNAi, pANDA-BAK1 dan pANDA-ETC; telah dibina. Vektor-vektor ini bersama satu vektor pengzahiran gene berlebihan yang sedia ada, iaitu pMDC32-ETC telah ditransform ke dalam padi MR219 menggunakan kaedah perantaraan *Agrobacterium*. Selepas transformasi tumbuhan, perubahan fenotip dan molekul atas tanaman padi transgenik telah dianalisis. Tanaman padi transgenik yang menghasilkan *OsI-BAK1* berlebihan menunjukkan fenotip daun berkedut dan berpintal pada peringkat awal pertumbuhan. Analisis histologi daun menunjukkan susunan sel dalam tisu vaskular yang tidak teratur berbanding dengan tumbuhan tidak ditransform. Sel-sel bulliform didapati bersaiz besar dan terbenam di dalam epidermis daun yang berkemungkinan menyebabkan ketidaknormalan morfologi pada bahagian daun. Perendam gen *OsI-BAK1* dalam padi menghasilkan bilangan biji padi yang hijau dan tidak berisi iaitu dua kali ganda tinggi berbanding dengan tumbuhan yang tidak ditransformkan. Analisis histologi telah dijalankan untuk

mengkaji anatomi biji, batang dan daun padi *Os*I-BAK1RNAi. Keratan melintang biji padi menunjukkan bahawa biji tersebut tidak mempunyai embrio atau mempunyai embrio yang perkembangannya terbantut. Secara keseluruhannya, gen *OsI-BAK1* mungkin memainkan peranan yang penting dalam proses perkembangan biji dan susunan sel daun. Penghasilan *OsI-ETC* yang berlebihan dalam padi menyebabkan kegagalan kalus embriogenik untuk menghasilkan pucuk. Ekspresi konstitutif *OsI-ETC* dalam padi mungkin meningkatkan pengeluaran etilena dan membantutkan regenerasi pucuk. Tanaman padi transgenik yang mengandungi RNAi konstruk untuk perendam gene *OsI-ETC* tidak menunjukkan perbezaan morfologi yang jelas berbanding dengan tumbuhan yang tidak ditransformkan. Ini mungkin disebabkan konstruk ini tidak berfungsi dan perendaman ekspresi gen tidak berlaku pada pokok *Os*I-ETCRNAi untuk memberi perubahan fenotip yang jelas. Analisis fungsi *OsI-BAK1* dan *OsI-ETC* dalam kajian ini telah memberi penjelasan mengenai fungsi biologi mereka dalam padi.

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