



UNIVERSITI PUTRA MALAYSIA

FUNCTIONAL ANALYSIS OF *PAL2* GENE PROMOTER IN *ARABIDOPSIS THALIANA* (L.) HEYNH. DURING PLANT DEVELOPMENT EXPOSED TO BIOTIC AND ABIOTIC STRESSES

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**MASTER OF SCIENCE
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By
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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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FUNCTIONAL ANALYSIS OF *PAL2* GENE PROMOTER IN *ARABIDOPSIS THALIANA* (L.) HEYNH. DURING PLANT DEVELOPMENT EXPOSED TO BIOTIC AND ABIOTIC STRESSES

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November 2011

Chairman : Mohd Puad Abdullah, PhD

Faculty : Biotechnology and Biomolecular Sciences

Phenylalanine ammonia-lyase (PAL; E.C.4.3.1.5) enzyme is essential for plant normal growth, development and adaptation to different environmental stresses. In *Arabidopsis thaliana*, the PAL enzyme is encoded by four gene isoforms which are designated as *PAL1* (AT2G37040), *PAL2* (AT3G53260), *PAL3* (AT5G04230), and *PAL4* (AT3G10340) respectively. *PAL1* and *PAL2* genes are closely related to each other phylogenetically and functionally. *PAL1* promoter is involved in plant development and also plant response under induction of a myriad of stresses. However, functional analysis on the *PAL2* promoter of *A. thaliana* has not been carried out. The *PAL2* promoter activities were investigated by fusing 1.8-kb 5' upstream of the translation start site with a β -glucuronidase (GUS) gene in transgenic

A. thaliana. The *PAL2* promoter activities were associated with the structural development of the plant and its organs and avirulent *Pseudomonas syringae* pv. *tomato* JL1065 induced the promoter response in an organ-specific manner. In the context of plant development, the *PAL2* promoter was active from the germination of young seedling to the reproductive stage, particularly in the rosette leaf, root, and inflorescence stem which are the major structural organs supporting the floral organs particularly bud, flower, and silique. The rosette leaf, root and stem are considered as the major structural organs as they provide the mechanical strength to support vertical position of the whole plant. The *PAL2* promoter activities in both rosette leaf and root were roughly 3-fold, and stem 2-fold higher than the floral organs and silique. The *PAL2* promoter activities displayed decreasing trend in the aerial organs with position further from the rosette leaves. In the context of plant adaptation, *PAL2* promoter activities was induced in the distal root with roughly 2-fold increase after 4-day post-inoculation with avirulent JL1065 in the aerial organs, suggesting *PAL2* promoter was involved in induced defence system. During moderate water deficit stress mediated by sodium chloride and polyethylene glycol solution for short-term period, *PAL2* promoter activities were not significantly induced for water stress-responsiveness. These findings imply that *PAL2* promoter maybe regulated transcriptionally for the normal plant structural development, and plant adaptation to avirulent *Pseudomonas* infection.

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

FUNGSI ANALISIS PROMOTER GEN *PAL2* DALAM PERTUMBUHAN *ARABIDOPSIS THALIANA* (L.) HEYNH. SEMASA TERDEDAH KEPADA STRES BIOTIK DAN ABIOTIK

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Enzim phenylalanine ammonia-lyase (PAL; E.C.4.3.1.5) adalah penting untuk perkembangan normal tumbuhan dan juga penyesuaian tumbuhan terhadap stres alam sekitar yang berlainan. Dalam *Arabidopsis thaliana*, enzim PAL dikod oleh empat isoform gen yang dinamakan sebagai *PAL1* (AT2G37040), *PAL2* (AT3G53260), *PAL3* (AT5G04230) dan *PAL4* (AT3G10340). *PAL1* dan *PAL2* adalah serupa dari segi filogenetik dan fungsi. Aktiviti promoter *PAL1* adalah terlibat dalam perkembangan tumbuhan dan juga respon tumbuhan semasa pengaruh pelbagai stres. Walau bagaimanapun, analisis fungsi terhadap aktiviti promoter *PAL2* belum dijalankan lagi. Aktiviti promoter *PAL2* dikaji dengan menggabungkan 1.8-kb 5' dari tapak permulaan translasi dengan gen β -

glucuronidase (*GUS*) dalam *A. thaliana* transgenik. Aktiviti promoter *PAL2* menunjukkan ia berkaitan dengan perkembangan struktur tumbuhan dan organnya, di samping boleh diaruh oleh avirulent *Pseudomonas syringae* pv. *tomato* JL1065 secara organ spesifik. Dalam konteks perkembangan tumbuhan, aktiviti promoter *PAL2* adalah aktif dari percambahan anak benih hingga peringkat reproduktif, terutamanya daun roset, akar, dan batang infloresen merupakan organ struktur yang utama menyokong organ bunga terutamanya tunas, bunga, dan silique. Roset, akar, dan batang dianggap sebagai organ struktur utama kerana mereka memberikan kekuatan mekanikal untuk menyokong kedudukan menegak seluruh tumbuhan. Aktiviti promoter *PAL2* dalam roset dan akar adalah kira-kira 3 kali ganda, sementara batang adalah 2 kali ganda lebih tinggi daripada organ bunga dan silique. Aktiviti promoter *PAL2* menunjukkan penurunan trend dalam organ arial dengan kedudukan jauh dari daun roset. Dalam konteks mekanisme pertahanan, aktiviti promoter *PAL2* adalah teraruh di akar berjauhan dengan kira-kira 2 kali ganda lebih tinggi berikutan 4 hari selepas-inokulasi dengan patogen avirulent JL1065 di organ arial mencadangkan bahawa promoter *PAL2* adalah terlibat dalam sistem pertahanan. Dalam stres defisi-air sederhana yang teraruh oleh sodium chloride dan polyethylene glycol untuk tempoh masa pendek, aktiviti promoter *PAL2* tidak teraruh secara ketara untuk tindak balas tumbuhan terhadap stres air. Penemuan ini menunjukkan bahawa aktiviti promoter *PAL2* adalah dikawal secara transkripsi untuk perkembangan normal struktur tumbuhan, dan juga penyesuaian tumbuhan terhadap jangkitan avirulent *Pseudomonas*.

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I certify that a Thesis Examination Committee has met on 18 November 2011 to conduct the final examination of Wong Jeh Haur on his thesis entitled "**Functional Analysis of *PAL2* Gene Promoter in *Arabidopsis thaliana* (L.) Heynh. During Plant Development Exposed to Biotic and Abiotic Stresses**" in accordance with the Universities and University Colleges 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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