UNIVERSITI PUTRA MALAYSIA

FUNCTIONAL ANALYSIS OF ARABIDOPSIS THALIANA 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE GENE IN RESPONSE TO LIMITED WATER

SIGIT ISMAWANTO

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By

SIGIT ISMAWANTO

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Chairman : Assoc. Prof. Dr Mohd Puad Abdullah

Faculty : Biotechnology and Biomolecular Sciences

Prolonged drought conditions pose a serious threat to plant growth and productivity due to water limitation. To survive, plants adapt by altering various biochemical pathways leading to physiological changes appropriate for growth under water-limited condition. One chemical known to be induced during this condition is ethylene, a plant hormone that affects plant vegetative and reproductive behaviours. Ethylene is synthesized by a two-step biosynthetic pathway mediated by the enzyme 1-aminocyclopropane-1- carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO). In plants, both genes are members of separate gene families. Recent data have shown that members of the *ACO* and *ACS* gene families were affected by limited water condition in *Arabidopsis thaliana*. The involvement of the other members of the *ACC* and *ACO* gene families is unknown. Therefore, the

objectives of this study were to determine the *ACS* and *ACO* genes responding to water-limited stress condition in *Arabidopsis* plants and to functionally analyse the role of an inducible *ACO* gene in *Arabidopsis* plant during development and water-limited stress. The over-expression construct was generated by using the Gateway technology and introduced into *Arabidopsis* by the *Agrobacterium*-mediated floral dip method. From nine *AtACS* genes, only *AtACS2* and *AtACS6* responded to the PEG-induced water stress. *AtACS2* was induced in roots whereas *AtACS6* was down-regulated in leaves and roots. All six members of the *AtACO* gene family responded to PEG-induced water stress with four genes (*AtACO3*, *AtACO4*, *AtACO5* and *AtACO6*) were switched-off in roots whereas three genes (*AtACO1*, *AtACO3* and *AtACO6*) were induced in the leaves. Among the inducible *AtACO* genes, *AtACO1* was unique because this gene was not expressed in roots but induced in leaves. Over-expressing *AtACO1* in *Arabidopsis* plant changed the overall vegetative growth of the plant, particularly the root system where the root became shorter and lesser. Interestingly, under PEG-induced water stress the number of lateral roots was increased. Overall, the transgenic plants responded the same way as wild plants judging by the biochemical and physiological parameters associated with drought-stressed plants obtained from the plants. In short, the *AtACO1* gene was more likely involved in plant development particularly the root system.

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

FUNGSI ANALISIS GEN 1-AMINOCYCLOPROPANE -1-KARBOSILIK ASID PADA *ARABIDOPSIS THALIANA* DI BAWAH KEADAAN KEKURANGAN AIR

Oleh

SIGIT ISMAWANTO

Julai 2013

Pengerusi : Assoc. Prof. Dr. Mohd Puad Abdullah

Fakulti : Bioteknologi dan Sains Biomolekul

Keadaan kemarau berpanjangan menimbulkan ancaman yang serius kepada pertumbuhan dan produktiviti tumbuhan akibat kekurangan air. Untuk terus hidup, tumbuh-tumbuhan menyesuaikan diri dengan mengubah pelbagai tapakjalan biokimia yang membawa kepada perubahan fisiologi yang sesuai untuk berkembang di bawah keadaan air yang terhad. Salah satu bahan kimia yang diketahui teraruh semasa keadaan ini adalah etilena, hormon tumbuhan yang mempengaruhi perlakuan tumbuhan peringkat vegetatif dan reproduktif. Etilena disintesis oleh tapakjalan biosintesis dua-langkah yang dimangkinkan oleh enzim 1-aminocyclopropane-1-carboxylic acid sintase (ACS) dan 1-aminocyclopropane-1- carboxylic acid oksidase (ACO). Dalam tumbuhan, kedua-dua gen ini adalah ahli famili gen yang berasingan. Data terkini menunjukkan bahawa ekspresi satu gen daripada famili *ACO* dan *ACS*

terjejas oleh keadaan air yang terhad dalam tumbuhan *Arabidopsis thaliana*. Penglibatan ahli-ahli lain dalam famili gen *ACC* dan *ACO* tidak diketahui. Oleh itu, objektif kajian ini adalah untuk menentukan gen *ACS* dan *ACO* yang bertindak balas kepada keadaan air yang terhad dalam tumbuhan *Arabidopsis* dan untuk menganalisis peranan gen *ACO* boleh-aruh dalam tumbuhan tersebut semasa proses perkembangan dan di bawah keadaan kekurangan air. Binaan gen untuk pengekspresan-lebih *ACO1* telah dibangunkan dengan menggunakan teknologi Gateway dan diperkenalkan ke dalam *Arabidopsis* melalui kaedah celupan bunga berperantara-*Agrobacterium*. Daripada sembilan gen *AtACS,* hanya *AtACS2* dan *AtACS6* bertindak balas terhadap kekurangan air aruhan-PEG. *AtACS2* teraruh dalam akar manakala ekspresi *AtACS6* menurun dalam daun dan akar. Kesemua enam ahli famili gen *AtACO* bertindak balas terhadap keadaan kekurangan air aruhan-PEG dengan ekspresi empat gen (*AtACO3, AtACO4, AtACO5* dan *AtACO6*) dimatikan di akar manakala tiga gen (*AtACO1, AtACO3* dan *AtACO6*) diaruh atau ditingkatkan dalam daun. Di antara gen *AtACO* yang teraruh, *AtACO1* adalah unik kerana gen ini tidak diekspresi di akar tetapi diaruh dalam daun. Pengekspresan secara berlebihan gen *AtACO1* dalam tumbuhan *Arabidopsis* mengubah keseluruhan pertumbuhan vegetatif tumbuhan, terutamanya sistem akar di mana ianya menjadi lebih pendek dan kurang. Menariknya, di bawah keadaan kekurangan air bilangan akar sisi meningkat. Secara keseluruhannya, tumbuhan transgenik tersebut bertindak balas dengan cara yang hampir sama seperti tumbuhan liar berdasarkan ukuran parameter biokimia dan fisiologi berkaitan kemarau yang diperolehi. Secara ringkas, gen *AtACO1* berkemungkinan terlibat dalam perkembangan tumbuhan terutamanya sistem akar.

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I certify that a Thesis Examination Committee has met on 19 July 2013 to conduct the final examination of Sigit Ismawanto on his thesis entitled "Functional Analysis of Arabidopsis thaliana 1-Aminocyclopropane-1-Carboxylic Acid Oxidase Gene in Response to Limited Water" 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.

Members of the Thesis Examination Committee were as follows:

Janna Ong binti Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Noor Azmi Shaharuddin, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Ho Chai Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Sreeramanan Subramaniam, PhD
Senior Lecturer
Universiti Sains Malaysia
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Puad Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Suhaimi Napis, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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.

SIGIT ISMAWANTO

Date: 19 July 2013

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