UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL, PHYTOCHEMICAL AND PROTEOMIC RESPONSES OF *ANDROGRAPHIS PANICULATA* NEES. TO SALT STRESS

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By

DARYUSH TALEI

Thesis submitted to the School of Graduate Students, Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

August 2012

DEDICATIONS

بسم الله الرحمن الرحيم

This Thesis is Special Dedicated to

My Wife, Fatemeh Zaeromali and

My children Sarina and Karina

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Mohd Puad Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Andrographis paniculata Nees. is a medicinal plant belonging to the family Acanthaceae. The plant is important due to its pharmaceutical curative compound called diterpenoids. The compound is extracted from vegetative parts of the plant which was cultivated in the field from seeds. However, growing the plant is challenging especially in arid and semi-arid regions where salt concentration is relative high. In different parts of the world, the plant exists in various accessions but none of them was properly characterized for its level of salt tolerance. Therefore, the present study was conducted to determine the salt tolerance thresholds of various accessions of the plant, and finally, to characterize the accessions for their physiological, phytochemical and proteomic responses to elevated levels of salt. Salt tolerance thresholds of the various accessions of the plant were determined by exposing 6 of the accessions to different salt concentrations and exposure times at seedling. The experiments were carried out in a split split plot arrangement based on the RCBD. Based on salt tolerance threshold results various dependent parameters related to physiological, biochemical and proteomic responses of the 32 different accessions of the plant were evaluated at seedling and mature stages. Changes in metabolites and proteomes were monitored by using HPLC and 2D-polyacrylamide gel electrophoresis, respectively. It was found that the tolerance threshold of the plant was 12 dSm^{-1} for 15-day of exposure time. Based on the salt tolerance index of individual accession, the plant could be grouped into salt-tolerant, moderately salt-tolerant and salt-sensitive accessions. When exposed to a high salt concentration, the tolerant accessions accumulated higher concentrations of proline, K^+, Ca^{2+} and andrographolide, and lower concentration of Na^+ than the sensitive ones, suggesting a salt tolerance response dictated by metabolite and ion adjustments. However, the levels of photosynthetic parameters were decreased in all of the accessions, which indicate that the salt stress directly reduced photosynthesis in the plant. Andrographolide content was positively correlated with salt concentration; the higher concentration of the salt the larger increase in andrographolide was observed. The concentration of three main phytochemicals (andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide) evaluated were also positively correlated with salt tolerance index and were negatively correlated with proline content. Overall, these patterns resemble the plant's salt tolerance responses based on physiological and phytochemical performances. At proteomic level, the tolerant accessions showed dissimilar SDS-PAGE banding patterns of soluble leaf protein of the plant exposed to high salt concentrations. These proteins were further analyzed by 2D gel electrophoresis in combination with a spot protein analysis by using the PDQuest software. The analysis led to the detection of 32 induced proteins and 12 up-regulated proteins in the leaf and root of the salt-treated plants. Of the 44 detected proteins, 12 were sequenced with three of them matched superoxide dismutase, ascorbate peroxidase and ribulose-1, 5-bisphosphate oxygenase whereas the rest were unknown. The three known proteins are generally associated with plant response to environmental stresses and could represent general stress proteins in the present study. Together, the proteomic data and the diverse physiological and phytochemical reactions demonstrated by the different accessions of the

plant to salt stress would potentially be used as salt tolerance indicators in the plant's breeding program for salt tolerance improvement.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazzah Doktor Falsafah

RESPONS MORFOLOGI, FITOKIMIA DAN PROTEOMIK DALAM *ANDROGRAPHIS PANICULATA* NEES. TERHADAP TEKANAN GARAM

Oleh

DARYUSH TALEI

Ogos 2012

Pengerusi: Prof. Madya Mohd Puad Abdullah, PhD

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Andrographis paniculata Nees. adalah tumbuhan ubatan dari famili Acanthaceae.Tumbuhan itu adalah penting disebabkan sebatian kuratif farmaseutikal yang dipanggil diterpenoid. Sebatian ini diekstrak dari bahagian-bahagian vegetatif tumbuhan tersebut yang lazimnya ditanam di ladang melalui bijibenih. Walau bagaimanapun, menanam tumbuhan tersebut terutamanya di kawasan gersang dan separa-gersang adalah mencabar disebabkan kepekatan garam yang tinggi di kawasan tersebut. Di berbagai bahagian dunia, tumbuhan ini wujud dalam pelbagai aksesi tetapi tiada satupun daripada aksesi berkenaan yang telah dicirikan dari segi ketoleranan terhadap garam. Oleh itu, kajian ini telah dijalankan untuk menentukan ambang toleransi garam pelbagai aksesi tumbuhan tersebut, dan akhirnya untuk mencirikan aksesi tersebut dari segi respons fisiologi, fitokimia dan proteomik apabila didedahkan kepada garam pada kepekatan tinggi. Ambang toleransi garam bagi pelbagai aksesi tumbuhan tersebut ditentukan dengan mendedahkan 6 aksesi kepada kepekatan garam yang berbeza dan kali pendedahan pada peringkat anakbenih. Eksperimen ini telah dijalankan dalam pengaturan plot pecahan pecahan yang berdasarkan rekabentuk blok lengkap (RCBD). Berdasarkan keputusan ambang toleransi garam pelbagai parameter berkaitan dengan respons fisiologi, biokimia dan proteomik tumbuhan tersebut telah dinilai terhadap 32 aksesi berbeza pada peringkat anak benih dan matang. Perubahan metabolit dan proteom telah dipantau dengan menggunakan HPLC dan gel elektroforesis dua dimensi, masing-masing. Didapati ambang toleransi aksesi tumbuhan tersebut adalah pada 12 dSm^{-1} selama 15 hari masa pendedahan. Berdasarkan kepada indeks toleransi garam setiap aksesi, tumbuhan tersebut boleh dikumpulkan ke dalam kumpulan toleran garam, toleran sederhana garam dan sensitif garam. Apabila terdedah kepada kepekatan garam yang tinggi, aksesi toleran garam menunjukkan kepekatan prolina, K^+, Ca^{2+} dan andrografolida yang lebih tinggi dan kepekatan Na^+ yang lebih rendah berbanding dengan aksesi sensitif garam, menunjukkan respons toleransi garam yang didominasi oleh pelarasan pelbagai metabolit dan ion. Walau bagaimanapun, tahap parameter fotosintesis telah menurun dalam semua aksesi yang diuji, menunjukkan bahawa tekanan garam memberi kesan buruk kepada proses fotosintesis tumbuhan berkenaan. Kandungan andrografolida pula berkorelasi secara positif dengan kepekatan garam, semakin tinggi kepekatan garam yang digunakan semakin besar pula perubahan penghasilan andrografolida yang diperolehi.Kepekatan tiga fitokimia utama yang lain (andrografolida, neoandrografolida dan deoksi 14-11, 12-didehidroandrografolida) juga berkorelasi positif dengan indeks toleransi garam tetapi berkorelasi negatif dengan kepekatan prolina. Secara keseluruhannya, pola perubahan berkenaan menyerupai respons toleransi garam bagi tumbuhan tersebut berdasarkan kepada prestasi fisiologi dan fitokimia. Pada peringkat proteomik pula, aksesi toleran yang didedahkan kepada kepekatan garam yang tinggi menunjukkan perbezaan dari segi corak SDS-PAGE protein daun. Melalui kombinasi elektroforesis gel 2D dan analisis spot protein dengan menggunakan perisian PDQuest, analisis tersebut telah membawa kepada pengesanan 32 protein teraruh dan 12 protein yang ditingkat-kawal di dalam daun dan akar tumbuhan yang dirawat dengan garam. Daripada 44 protein yang dikesan, 12 daripadanya telah dijujukkan dengan tiga daripada mereka sepadan

dengan superoksida dismutase, askorbat peroksidase dan ribulose-1, 5-bifosfat oksigenase manakala selebihnya adalah tidak diketahui. Secara bersama, data proteomik dan pelbagai respons fisologi dan fitokimia yang ditunjukkan oleh pelbagai aksesi tumbuhan tersebut terhadap tekanan garam berpotensi untuk digunakan sebagai petunjuk toleransi garam dalam program pembaik-bakaan tumbuhan berkenaan terutamanya dari segi pembaikan tahap toleransi garam.

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I certify that a Thesis Examination Committee has met on 9 August to conduct the final examination of Daryush Talei on his Doctor of Philosophy thesis entitled “Morphological, Phytochemical and Proteomic Responses of *Andrographis paniculata* Nees. To Salt Stress” 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 degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. 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.

DARYUSH TALEI

Date: 9 August 2012

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