

ISOLATION, CHARACTERIZATION AND
EXPRESSION OF HEAT-SHOCK PROTEIN 90
GENE (*HSP90*) FROM *CRYPTOCORYNE CILIATA*
CULTURES

ZAIRUL FAZWAN MD ZAINORDIN

MASTER OF SCIENCE
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Main Supervisor: Associate Professor Azis Ahmad, Ph.D.

Co-supervisor: Associate Professor Che Yee Sun, Ph.D.

Faculty: Faculty of Science and Technology

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ISOLATION, CHARACTERIZATION AND EXPRESSION OF HEAT-SHOCK PROTEIN 90 GENE (*HSP90*) FROM *CRYPTOCORYNE CILIATA* CULTURES

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Main Supervisor : Associate Professor Aziz Ahmad, Ph.D.
Co-Supervisor : Associate Professor Cha Thye San, Ph.D.
Faculty : Faculty of Science and Technology

In the expansion of agriculture land for food production, salinity problem has led to huge losses in terms of arable land and productivity. As most of the economically important crop species are very sensitive to soil salinity, it is appropriate to study how plants respond to salinity stress. Heat-shock protein 90 (Hsp90) is an abundant and highly conserved molecular chaperones which assist other proteins in achieving proper folding or re-folding of stress-denatured polypeptides and involved in regulation of many essential cellular pathways towards environmental stress. In the present study, the *Hsp90* corresponding genes of a halophyte plant, *Cryptocoryne ciliata* were isolated using multiplex-PCR, 3'-RACE and 5'-RACE approaches. The gene were then characterized and expressed using qPCR technique. Three isoforms of cytosolic *Hsp90* genes were successfully isolated from *in vitro* plantlet: pPutative 1 (270 bp), partial-length *CcHsp90-1* (GenBank accession number: GU441770) and full-length *CcHsp90-2* (GenBank accession number: JN120021). Characterization of the genes showed that the *CcHsp90-1* partial-length cDNA (1570 nucleotides) encoded for a 420 amino acids with predicted molecular mass of 48.13 kDa,

consisting of two domains: middle-client protein interacting domain (1 to 260) and C-terminus dimerization domain (261 to 420). On the other hand, the full-length *CcHsp90-2* cDNA (2465 nucleotides) encoding 700 amino acids with predicted molecular mass of 79.95 kDa, consists of three domains: N-terminus ATP-binding domain (1 to 211), middle-client protein interacting domain (281 to 540) and C-terminus dimerization domain (541 to 700); the N-terminus and middle domain was linked by a charged linker domain (212 to 280). These CcHsp90 proteins shared high sequence homology (83 to 93%) as compared to other plant cytosolic Hsp90, but shared lower sequence homology (33 to 45%) compared to organelle and endoplasmic reticulum specific Hsp90 isoforms. The deduced CcHsp90 amino acid sequences possesses five-conserved amino acid signature sequence motifs characteristic of the Hsp90 family and a C-terminus MEEVD penta-peptide characteristic of the cytosolic Hsp90 isoform. The predicted quaternary architecture structure for CcHsp90-2 protein generated through molecular modeling was globally akin to yeast Hsp90. The expression analysis of the *CcHsp90* genes using qPCR shows that the relative expression of *CcHsp90-1* and *CcHsp90-2* genes were significantly upregulated with optimum expression of 5.66 and 8.94 fold, respectively, in response to salinity stress. This study provides baseline information to understand the role of CcHsp90 proteins adaptation of plants to salinity stress.

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PEMENCILAN, PENCIRIAN DAN PENGEKSPRESAN GEN BAGI 'HEAT-SHOCK PROTEIN 90' (*HSP 90*) DARIPADA KULTUR *CRYPTOCORYNE CILIATA*

ZAIRUL FAZWAN MD ZAINORDIN

Jun 2012

Penyelia Utama : Prof. Madya Aziz Ahmad, Ph.D.
Penyelia Bersama : Prof. Madya Cha Thye San, Ph.D.
Fakulti : Fakulti Sains dan Teknologi

Dalam pengembangan tanah pertanian untuk penghasilan makanan, masalah kemasinan menyebabkan kehilangan besar tanah subur dan penurunan produktiviti. Memandangkan kebanyakan tanaman penting sensitif terhadap tekanan kemasinan tanah, adalah perlu untuk mengkaji bagaimana tumbuhan bertindak balas terhadap tekanan ini. Protein heat-shock 90 (*Hsp90*) merupakan molekul protein chaperone terpelihara yang dihasilkan dengan banyak, bagi membantu protein-protein lain mencapai konfigurasi "lipatan" yang betul atau melipat semula polipeptida rosak dan terlibat dalam banyak aturan jaringan selular yang bertindakbalas terhadap tekanan persekitaran. Di dalam kajian ini, gen *Hsp90* daripada tumbuhan halophyta iaitu *Cryptocoryne ciliata* telah dipencilkan menggunakan kaedah multiplex-PCR, 3'-RACE dan 5'-RACE. Gen ini kemudiannya dicirikan dan diekspreskan menggunakan teknik qPCR. Tiga isoform gen *Hsp90* sitosolik berjaya dipencilkan daripada kultur *Cryptocoryne ciliata*: pPutative 1 (270 bp), *CcHsp90-1* (nombor akses GenBank: GU441770) dan *CcHsp90-2* (nombor akses GenBank: JN120021). Pencirian gen menunjukkan cDNA separa-panjang bagi gen *CcHsp90-1* (1570

nukleotida) yang mengkodkan 420 asid amino dengan anggaran jisim molekul seberat 48.13 kDa, terdiri daripada dua domain: domain pertengahan-interaksi protein pelanggan (1 hingga 260) dan domain terminal-C pendimeran (261 hingga 420). Manakala cDNA panjang-penuh bagi gen *CcHsp90-2* (2465 nukleotida) mengkod 700 asid amino dengan anggaran jisim molekul seberat 79.95 kDa, terdiri daripada tiga domain: domain terminal-N pengikatan ATP (1 hingga 211), domain pertengahan-interaksi protein pelanggan (281 hingga 540) dan domain terminal-C pendimeran (541 hingga 700); domain terminal-N dan pertengahan disambungkan dengan domain penyambung bercas (212 hingga 280). Kedua-dua protein CcHsp90 ini mempunyai kesamaan jujukan yang tinggi dengan protein Hsp90 sitosol tumbuhan yang lain (83 hingga 93%), tetapi mempunyai kesamaan jujukan yang sangat rendah dengan isoform Hsp90 khusus untuk organel dan retikulum endoplasmik (33 hingga 45%). Secara keseluruhannya, jujukan asid amino CcHsp90 mempunyai lima motif terpelihara keluarga Hsp90 beserta peptida MEEVD iaitu ciri khusus pada terminal-C isoform Hsp90 sitosol. Ramalan struktur rekabentuk bagi protein CcHsp90-2 didapati menyerupai model protein Hsp90 yis. Analisis pengekspressan gen *CcHsp90* menggunakan qPCR menunjukkan bahawa kadar pengekspressan relatif gen *CcHsp90-1* dan *CcHsp90-2* menunjukkan peningkatan hiliran yang ketara dengan ekspresi optima sebanyak 5.66 dan 8.94 kali ganda masing-masing, sebagai tindakbalas terhadap tekanan kemasinan. Kajian ini dapat memberikan maklumat asas untuk memahami peranan protein CcHsp90 dalam penyesuaian terhadap tekanan kemasinan.