

Transient Expression Analysis of Putative W-Box Element From *Elongation Factor-1 α* (*MeEF1 α 2*) Gene Promoter In Tobacco (*Nicotiana tabacum* L.)

Student: Armelia Aprilianti Melkias

Thesis (2010), Master's program In Biotechnology, School of Life Sciences and Technology-ITB, email: armelia_melkias@yahoo.com

Advisors: Dr. Sony Suhandono

School of Life Sciences and Technology ITB, email: sony@sith.itb.ac.id

Degree: Magister Sains (M.Si), Conferred July 2010

Abstract

Elongation Factor-1 α (*EF-1 α*) is an essential elongation factor for protein synthesis in eukaryotic. It catalyzes binding of aminoacyl-tRNA to the A site ribosome. In the previous study, one of the *EF-1 α* gene had been isolated and its promoter characterized (*MeEF1 α 2*). Based on preliminary bioinformatics analysis in this study, *MeEF1 α 2* promoter had a putative W-Box (AGTCA) that was present at -50 pb to -54 pb from TSS (Transcription Start Site). Thus, the aim of this study is to investigate the role of putative W-Box by site-directed mutagenesis and to analyze the mutation effect in tobacco (*Nicotiana tabacum* L.). PCR (Polymerase Chain Reaction) method using mismatch oligonucleotide was used to create two point mutations (A and T). Non-mutated promoter (*MeEF1 α 2*⁺) and mutated promoter (*MeEF1 α 2*⁻) were fused to *gusA* reporter gene and cloned to pCAMBIA 1303. These vectors were introduced to tobacco mediated by *Agrobacterium tumefaciens* GV3101. Activities of the promoters were detected by *gusA* reporter gene in tobacco using histochemical staining. The results showed that *gusA* expression regulated by *MeEF1 α 2*⁻ promoter (64,27%) was higher than *gusA* expression was regulated by *MeEF1 α 2*⁺ promoter (44,78%) in non-wounded tobacco. Furthermore, *gusA* expression in wounded tobacco (23,92%) was less than *gusA* expression in non-wounded tobacco (44,78%) that it was regulated by *MeEF1 α 2*⁺ promoter. Therefore, putative W-Box in *MeEF1 α 2* promoter plays as motif-repressor binding in non-wounded tissue. Expression of *gusA* regulated by *MeEF1 α 2*⁻ promoter almost equal with *gusA* activity regulated by *MeEF1 α 2*⁺ promoter in response to wounding. Thus, mutation of putative W-Box from *MeEF1 α 2* promoter did not give significant effect on *gusA* expression.

Key words: *MeEF1 α 2* promoter, mutation of putative W-Box, transient expression, motif-repressor binding

Analisis Ekspresi Elemen W-Box Putatif dari Promoter Gen *Elongation Factor-1 α* (*MeEF1 α 2*) pada Tembakau (*Nicotiana tabacum* L.) secara Transien

Mahasiswa: Armelia Aprilianti Melkias

Tesis (2010), Program Studi Magister Bioteknologi SITH, email: armelia_melkias@yahoo.com

Pembimbing: Dr. Sony Suhandono

SITH-ITB, email: sony@sith.itb.ac.id

Gelar: Magister Sains (M.Si), Wisuda Juli 2010

Abstrak

Elongation Factor-1 α (*EF-1 α*) merupakan faktor elongasi dalam sintesis protein pada organisme eukariot. Protein *EF-1 α* mengkatalisis pengikatan aminoasil-tRNA ke sisi akseptor ribosom. Penelitian sebelumnya berhasil mengkarakterisasi promoter gen *EF-1 α* (*MeEF1 α 2*). Pada studi pendahuluan penelitian ini ditemukan W-Box putatif (AGTCA) yang terletak pada -50 pb s.d -54 pb dari TSS (*Transcription start site*). Oleh karena itu, penelitian ini bertujuan untuk mengetahui peranan W-Box putatif dengan cara melakukan mutasi serta mengamati efek mutasi tersebut secara transien pada tembakau. Mutasi dilakukan dengan metode PCR (*Polymerase Chain Reaction*) menggunakan sepasang primer, dengan primer *forward* yang mengandung mutasi pada dua titik (A dan T). Promoter yang tidak dimutasi (*MeEF1 α 2⁺*) dan dimutasi (*MeEF1 α 2⁻*) difusikan dengan *gusA* sebagai gen reporter di dalam pCAMBIA 1303, serta ditransformasi ke dalam *Agrobacterium tumefaciens* GV3101 untuk kemudian dianalisis ekspresi transiennya dalam tembakau. Hasil uji ekspresi transien menunjukkan ekspresi *gusA* yang diregulasi oleh promoter *MeEF1 α 2⁻* (64,27%) lebih tinggi daripada promoter *MeEF1 α 2⁺* (44,78%) pada tembakau utuh. Saat tembakau dilukai, ekspresi *gusA* yang dikendalikan oleh promoter *MeEF1 α 2⁺* (23,92%) lebih rendah daripada ekspresi *gusA* pada tembakau utuh (44,78%). Hal ini menunjukkan bahwa W-Box putatif pada promoter *MeEF1 α 2* berperan sebagai motif pengikatan bagi represor ketika jaringan tembakau tidak terluka. Ekspresi *gusA* yang diregulasi oleh promoter *MeEF1 α 2⁻* hampir sama dengan ekspresi *gusA* pada promoter *MeEF1 α 2⁺* pada tembakau yang tidak dilukai. Oleh karena itu, mutasi pada W-Box putatif dari promoter *MeEF1 α 2* tidak memberikan efek yang signifikan terhadap ekspresi *gusA* ketika tembakau dilukai.

Kata kunci: promoter *MeEF1 α 2*, mutasi W-Box putatif, uji ekspresi transien, represor