Metal inducible activity of the oil palm metallothionein-like gene promoter (MT3-A) in prokaryotes.

ABSTRACT

Reporter gene activity under the regulation of the oil palm metallothionein-like gene, MT3-A promoter was assessed in prokaryotes. Vector constructs containing MT3-A promoter with (W1MT3-A) and without (W2MT3-A) five prime untranslated region (5'-UTR) fused to βglucuronidase (GUS) gene in pCAMBIA 1304 vector were produced. 5'-rapid amplification of cDNA ends (RACE) using mRNA isolated from Escherichia coli and Agrobacterium tumefaciens harboring W1MT3-A confirmed that fusion transcripts of MT3-A 5'-UTR-GUS were successfully produced in both bacteria. Competitive PCR and GUS fluorometric assay showed changes in the level of GUS gene transcripts and enzyme activity in response to increasing concentrations of Cu²+ and Zn²+. The application of Cu²+ increased GUS activity and GUS mRNA level in both bacteria. In E. coli, a high level of GUS activity driven by W1MT3-A and W2MT3-A was observed in treatment with 25 µM Cu²⁺ resulting in an increase in the GUS mRNA level to 7.2 and 7.5 x 10^{-4} pmol/µl respectively, compared to the control (5.1 x 10⁻⁴ pmol/µl). The lowest GUS activity and GUS mRNA level were obtained for W1MT3-A and W2MT3-A in the presence of 100 µM Cu²+ in both bacteria compared to the control (without Cu²+). The application of different Zn²+ concentrations resulted in a strong decrease in the GUS activity and GUS mRNA level in E. coli and A. tumefaciens. These findings showed that the oil palm MT3-A promoter is functional in prokaryotes and produced detectable GUS transcripts and enzyme activities. This promoter may potentially be used in prokaryotic systems which require metal inducible gene expression.

Keyword: Metallothionein-like gene promoter; Elaeis guineensis; Escherichia coli.