Identification and characterisation of thiamine pyrophosphate (TPP) riboswitch in Elaeis guineensis

ABSTRACT

The oil palm (Elaeis guineensis) is an important crop in Malaysia but its productivity is hampered by various biotic and abiotic stresses. Recent studies suggest the importance of signalling molecules in plants in coping against stresses, which includes thiamine (vitamin B1). Thiamine is an essential microelement that is synthesized de novo by plants and microorganisms. The active form of thiamine, thiamine pyrophosphate (TPP), plays a prominent role in metabolic activities particularly as an enzymatic cofactor. Recently, thiamine biosynthesis pathways in oil palm have been characterised but the search of novel regulatory element known as riboswitch is yet to be done. Previous studies showed that thiamine biosynthesis pathway is regulated by an RNA element known as riboswitch. Riboswitch binds a small molecule, resulting in a change in production of the proteins encoded by the mRNA. TPP binds specifically to TPP riboswitch to regulate thiamine biosynthesis through a variety of mechanisms found in archaea, bacteria and eukaryotes. This study was carried out to hunt for TPP riboswitch in oil palm thiamine biosynthesis gene. Riboswitch detection software like RiboSW, RibEx, Riboswitch Scanner and Denison Riboswitch Detector were utilised in order to locate putative TPP riboswitch in oil palm ThiC gene sequence that encodes for the first enzyme in the pyrimidine branch of the pathway. The analysis revealed a 192 bp putative TPP riboswitch located at the 3' untranslated region (UTR) of the mRNA. Further comparative gene analysis showed that the 92-nucleotide aptamer region, where the metabolite binds was conserved inter-species. The secondary structure analysis was also carried out using Mfold Web server and it showed a stem-loop structure manifested with stems (P1-P5) with minimum free energy of -12.26 kcal/mol. Besides that, the interaction of riboswitch and its ligand was determined using isothermal titration calorimetry (ITC) and it yielded an exothermic reaction with 1:1 stoichiometry interaction with binding affinities of 0.178 nM, at 30°C. To further evaluate the ability of riboswitch to control the pathway, exogenous thiamine was applied to four months old of oil palm seedlings and sampling of spear leaves tissue was carried out at days 0, 1, 2 and 3 post-treatment for expression analysis of ThiC gene fragment via quantitative polymerase chain reaction (qPCR). Results showed an approximately 5-fold decrease in ThiC gene expression upon application of exogenous thiamine. Quantification of thiamine and its derivatives was carried out via HPLC and the results showed that it was correlated to the down regulation of ThiC gene expression. The application of exogenous thiamine to oil palm affected ThiC gene expression, which supported the prediction of the presence of TPP riboswitch in the gene. Overall, this study provides the first evidence on the presence, binding and the functionality of TPP riboswitch in oil palm. This study is hoped to pave a way for better understanding on the regulation of thiamine biosynthesis pathway in oil palm, which can later be exploited for various purposes especially in manipulation of thiamine biosynthesis pathways in combating stresses in oil palm.