

Construction of PHB and PHBV multiple-gene vectors driven by an oil palm leaf-specific promoter

ABSTRACT

One of the targets in oil palm genetic engineering programme is the production of polyhydroxybutyrate (PHB) and polyhydroxybutyrate-co-valerate (PHBV) in the oil palm leaf tissues. Production of PHB requires the use of *phbA* (β -ketothiolase type A), *phbB* (acetoacetyl-CoA reductase) and *phbC* (PHB synthase) genes of *Ralstonia eutropha*, whereas *bktB* (β -ketothiolase type B), *phbB*, *phbC* genes of *R. eutropha* and *tdcB* (threonine dehydratase) gene of *Escherichia coli* were used for PHBV production. Each of these genes was fused with a transit peptide (Tp) of oil palm acyl-carrier-protein (ACP) gene, driven by an oil palm leaf-specific promoter (LSP1) to genetically engineer the PHB/PHBV pathway to the plastids of the leaf tissues. In total, four transformation vectors, designated pLSP15 (PHB) and pLSP20 (PHBV), and pLSP13 (PHB) and pLSP23 (PHBV), were constructed for transformation in *Arabidopsis thaliana* and oil palm, respectively. The phosphinothricin acetyltransferase gene (*bar*) driven by CaMV35S promoter in pLSP15 and pLSP20, and ubiquitin promoter in pLSP13 and pLSP23 were used as the plant selectable markers. Matrix attachment region of tobacco (RB7MAR) was also included in the vectors to stabilize the transgene expression and to minimize silencing due to positional effect. Restriction digestion, PCR amplification and/or sequencing were carried out to ensure sequence integrity and orientation.

Keyword: Multiple-gene vectors, Oil palm leaf-specific promoter, Plastids, Polyhydroxybutyrate (PHB), Polyhydroxybutyrate-co-valerate (PHBV), Single-gene vectors