

Cloning of amorpho-4,11-diene synthase (*ADS*) or cytochrome P450 monooxygenase genes (*CYP71AV1*) co-expressing with farnesyl pyrophosphate synthase (*FPS*) for transformation to *Artemisia annua* L. plant

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Abstract

Artemisinin is the most effective anti-malarial drug produced from *Artemisia annua* L. In this study, key enzyme genes, encoding amorpho-4,11-diene synthase (*ADS*) and cytochrome P450 monooxygenase (*CYP71AV1*) were constructed with or without co-expressing of farnesyl pyrophosphate synthase (*FPS*) gene and inserted into the multiple cloning site of pCAMBIA3300. Confirmations of these constructs were demonstrated by PCR, restriction analysis and nucleotide sequencing analysis. The results from PCR showed that cloned genes consist of 1,653 bp for *ADS* gene, 1,501 bp for *CYP71AV1* gene, 2,712 bp for *FPS-ADS* and 2,538 bp for *FPS-CYP71AV1* gene cassettes. Comparing of deduced polypeptide with NCBI database showed 99% identical to *FPS* (accession number GQ420346) *ADS* (accession number HQ315833) and *CYP71AV1* gene (accession number HQ315834). These constructs were transferred into *Artemisia annua* using *Agrobacterium tumefaciens* strain EHA105 in order to investigate artemisinin contents and terpenoid constituents. The putative transformants were obtained via direct shoot and callus formation regenerated from leaf-disc cultures and most of infected plants performed calli prior followed with regenerated shoots. Regenerated shoots were selected on bialaphos containing medium and confirmed for gene insertion. The PCR positive result of *bar* gene was detected after screened the putative transformed shoots that survived in selective media. These transgenic plants will be further confirmed by Southern blot hybridization prior to analyze for biochemical contents.