Abstract

Triterpenoid saponins are glycoside triterpenes in the saponin group that have significant medicinal properties that include anti-inflammatory, antibacterial, antifungal, and antioxidant activities. Abrusoside and glycyrrhizin, part of the triterpenoid saponins, are bioactive compounds that are produced in *Abrus precatorius*. Although it has the potential to play critical roles in multiple industries, the specific enzymes and biosynthesis pathway are still understudied. This study aims to identify and characterize CYP450 candidates differentially expressed in *A. precatorius* under combined hydroponic and MeJA stress for abrusoside/glycyrrhizin biosynthesis. Transcriptomics data was filtered to select CYP450 candidates followed by transcript expression level visualization using heatmap. Candidate gene characterization analysis was conducted by generation of phylogeny trees built using MEGA11, identification of conserved motifs using MEME-suite, and predicted protein 3D structures modelling using AlphaFold. Selected candidates based on the expression data and gene characterization were used for primer design for qualitative polymerase chain reaction (qPCR) analysis to further validate the transcriptomic data. Primer pairs were designed using Primer-BLAST/Primer3, first strand cDNA was synthesised, and PCR condition was optimized. A total of 88 CYP450s gene candidates were successfully identified from the transcriptomic data and heatmap analysis of these gene identify CYP450s genes that were highly upregulated by the combination of hydroponic-MeJA treatment. Phylogenetic/motif analyses indicated that CYP72A219-like, CYP84A1-like, -amyrin 28-monooxygenase-like, and NADPH-CPR and were found to cluster the closest with triterpenoid-oxidizing homologs of *Glycine max* and *Medicago truncatula*. Primer candidates were successfully obtained but still required further optimization to get the specific target gene for accurate qPCR analysis. Stress-responsive CYP450s in the triterpenoid biosynthesis of the product are in silico evidenced, and experimental validation of the studies will be possible by redesigned primers and RT-qPCR. This study was able to successfully validate in silico and transcriptomics analysis regarding cytochrome P450 gene expression patterns and motif analysis, but unable to successfully perform wet lab and requires improved methodology and strategies.

Keywords: *Abrus precatorius*, secondary metabolites, triterpenoid saponins, cytochrome p450, abrusoside, glycyrrhizin