Chapter 1

Introduction

1.1 Background

Glycyrrhizin and abrusoside are secondary metabolites found in *Abrus precatorius*. These triterpenoid saponins have gained traction recently due to their potential usage as herbal medicines and as low-calorie natural sweeteners. Its chemical structure combines a non-sugar molecule called aglycone and one or more sugar units called a glycone, which contributes to the sweetness that can range from 30 to 400 times sweeter than sucrose (Dwivedi, 2022; Timilsena, 2023). With this characteristic, both compounds have the potential to be used as sugar substitutes and flavoring modifiers in related industries. For instance, the dietary food and beverage industries, candies, herbs, and seasonings (Timilsena et al., 2023). These secondary metabolites are abundant in its leaves and roots, which play acrucial role in defending plants from various stress factors (Rogowska et al., 2025).

The initial biosynthesis step of both glycyrrhizin and abrusoside starts with a triterpenoid precursor molecule called -amyrin, which is catalyzed by cytochrome P450 enzymes (CYP450s). Through oxidative modifications in the initial triterpene skeleton, the formation of aglycone is the end-product of these enzymes that will later be continued to glycosylation processes (Seki et al., 2015). Most notable applications of CYP450 enzymes are in protein engineering and various synthetic biology approaches, such as fermentation in microbial cells to obtain valuable chemicals (Girvan and Munro, 2016). The role of CYP450 enzymes includes catalyzing various reactions such as fatty acid metabolism, steroid hormones, cholesterol synthesis, and secondary metabolites (Abdelmonem et al., 2024). By oxidizing hydrophobic compounds, CYP450s allow faster metabolism and excretion, which are essential in drug metabolism production (Gilani and Cassagnol, 2025). This metabolization capability of CYP450s also extends to xenobiotic hosts and clearance of toxic compounds.

However, the concentration of glycyrrhizin and abrusoside occurring in *A.precatorius* is very low under normal conditions. The natural yield of these triterpenoid saponins only reach 0.058–0.427% dry weight (DW), even in optimized untransformed cell cultures (Sambre et al., 2017). Therefore, increasing the yield of these saponins on the same amount of plants is the focus. Previous studies have utilized multiple strategies to increase the yield of these secondary metabolites, including utilizing different biotic and abiotic elicitors. One of them includes the usage of PEG (polyethylene glycol), CdCl2, cellulase, and mannan which boosted around 5.4 to 8.6-fold content in related species (*Glycyrhiza glabra*), increasing feasibility and potential in *A. precatorius* (Srivastava et al., 2018). This study has observed the impacts of hydroponic treatment and methyl jasmonate (MeJA) treatment on plant stress responses. Hydroponic treatment is a plant cultivation system where the plant roots are submerged in water, which exposes soil-adapted plants to abiotic stress. The form of this stress is oxidative stress, due to limited oxygen availability in the roots induced by the continual submersion in water (Manghwar, 2024). Whereas methyl jasmonate stimulates biotic stress conditions by acting as a plant signaling molecule that triggers gene expression patterns associated with secondary metabolite biosynthesis and stress defense, such as defense against herbivore organisms or physical injury (Divekar et al., 2022). These combinations of treatments potentially form unique and amplified transcriptional profiles that are not attainable with using either stressor alone, thus providing an ideal model for investigating stress-inducible gene regulation (Lowe et al., 2017). Our previous study sucessfully replicated the earlier study by combining hydroponic cultivation and MeJA treatment to increase accumulation of glycyrrhizin and abrusoside in *A. precatorius*. Transcriptomic data of *A. precatorius* samples were obtained (Istiandari et al. in press) identifying differential expression genes potentially involved in the biosynthesis of glycyrrhizin and abrusoside, such as the CYP450 genes.

1.2 Objective

This study aims to identify and characterize candidate cytochrome P450 (CYP450) genes differentially expressed in *A. precatorius* cultivated under hydroponic cultivation and treated with methyl jasmonate. To achieve this objective, the study conducted:

1. Transcriptome-based identification of CYP450 gene candidates that exhibit differential expression in response to MeJA treatment.
2. Phylogenetic analyses to determine evolutionary relationships between the identified candidates and previously characterised CYP450s associated with specialised metabolite biosynthesis.
3. Conserved protein motifs and functional domains identification related to abrusoside and glycyrrhizin biosynthetic pathways.
4. A specific primer design for quantitative PCR (qPCR) assays to enable further validation of selected candidate genes.

1.3. Hypothesis

- Null Hypothesis (H_o): The candidate genes with MeJA treatment does not trigger any significant difference in CYP450 gene expression in Abrus precatorius, and the identified CYP450 candidates neither share phylogenetically related specialised metabolite biosynthetic CYP450s nor have any conserved motifs or functional domains related to abrusoside or glycyrrhizin biosynthesis. Additionally, candidate genes are not uniquely or effectively amplified by designed primers in qPCR validation tests.

- Alternative Hypothesis (H_1): Candidate genes with MeJA treatment shows significant differential expression in specific CYP450 genes of *Abrus precatorius*, and the identified CYP450 candidates show strong phylogenetic relationships and conserved protein motifs has the characteristic roles in abrusoside or glycyrrhizin biosynthesis. Additionally, the designed primers specifically and efficiently amplify these candidate genes in qPCR validation assays.