Title: Construction of Enzymatic Kinetic Models for Anthocyanin Biosynthesis in Grapes

Starting date: December 2024-March 2025
Duration: 6 months
Salary: Standard salary provided by the Implanteus project
Site: Beijing, China
Institute and Department: Institute of Botany, Chinese Academy of Sciences, Grape quality regulation lab.

Background

Anthocyanins are important secondary metabolites in grapes, contributing significantly to the color and health benefits of grape products. The biosynthesis of anthocyanins involves a series of enzymatic reactions, each catalyzed by specific enzymes. Understanding the kinetic parameters of these enzymes is crucial for elucidating the regulatory mechanisms of anthocyanin biosynthesis and for developing strategies to enhance anthocyanin content in grapes. Despite extensive research, there is still a lack of comprehensive kinetic models that accurately describe the dynamic behavior of anthocyanin biosynthesis in grapes. We have already established a certain research foundation on the anthocyanin composition model, which has been published in Annals of Botany.

Objectives

1. To characterize the kinetic parameters of key enzymes involved in anthocyanin biosynthesis in grapes.
2. To construct a detailed enzymatic kinetic model that simulates the dynamic accumulation of anthocyanins in grape berries.
3. To validate the model using time-series data from different grape varieties and environmental conditions.
4. To identify potential bottlenecks and regulatory points in the anthocyanin biosynthesis pathway.

Methods

1. Enzyme Characterization: Key enzymes involved in anthocyanin biosynthesis, such as flavonoid 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT), will be purified and characterized. Kinetic parameters (Km, Vmax, Ki) will be determined using in vitro enzyme assays.
2. Model Construction: A detailed enzymatic kinetic model will be constructed using the kinetic parameters obtained from enzyme characterization. The model will simulate the dynamic accumulation of anthocyanins, taking into account the interactions between different enzymes and the influence of environmental factors.
3. Model Validation: The model will be validated using time-series data on anthocyanin composition from different grape varieties (e.g., 'Pinot noir', 'Sangiovese', 'Gamay Freaux', and 'Cabernet Sauvignon') grown under various environmental conditions. The model predictions will be compared with experimental data to assess its accuracy.
4. Bottleneck Identification: Sensitivity analysis will be performed to identify potential bottlenecks and regulatory points in the anthocyanin biosynthesis pathway. This will involve analyzing the

effects of changes in enzyme activities and environmental conditions on anthocyanin accumulation.

Related reference: Wang Y, Shang B, Génard M, Hilbert-Masson G, Delrot S, Gomès E, Poni S, Keller M, Renaud C, Kong J, Chen J, Liang Z, Dai Z. **2023**. Model-assisted analysis for tuning anthocyanin composition in grape berries. ***Annals of Botany*** 132, 1033-1050.

Pre-requirements:

All master 1 students major in plant science are welcomed. We look for a motivated candidate, with open personality, independent thinking and good skills of communication and writing. Skills in coding (e.g. with R) will be appreciated.

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The lab: The hosting lab is led by Prof. Zhanwu Dai, who has obtained his PhD diploma from Avignon University and then worked as a scientist (CR1) at INRAE for 9 years. With in total of 13 years of experience, Prof. Dai is internationally very well known for his work on grape quality regulation research with multidisciplinary approaches, including modeling, transcriptomes, metabolomes, epigenomes, as well as phenomes. The lab is conducting world-leading projects around grape quality at different levels and have extensive international collaborations with researchers from France, Spain, Italy, Germany, South African, Belgium, Australian, New Zealand, and USA. A list of Prof. Dai's publication could be found: https://www.researchgate.net/profile/Zhanwu-Dai/research