M2 INTERNSHIP SUBJECT – 6 months from January 2025

TITLE (in 120 characters): Spatiotemporal transcriptome changes occurring during graft union formation

CONTEXT: Human health and nutrition depends on the viability of grafted crops and therefore successful grafting. Grafting involves cutting the scion and rootstock, and maintaining the cut surfaces in close proximity. Graft union formation requires scion/rootstock adhesion, the construction of a common and functional cell wall at the graft interface, and the formation of vascular connections between the two partners. Currently, our understanding of grafting is limited by the lack of spatial knowledge of the processes occurring during graft union formation.

The genes differentially expressed during graft union formation have been described in bulk tissue samples of many plant species (Feng et al., 2024; Loupit et al., 2023a). However, plant stems consist of many cell and tissue types that have different transcript, protein and metabolite profiles and different responses to stimuli hence bulk tissue approaches provide only limited information. We have recently published the first spatial metabolomics study of the graft interface of any species (Loupit et al., 2023b). We found that stilbenes accumulate in the wounded xylem parenchyma tissues, whereas naringenin accumulates in the newly formed callus tissues. This pattern of stilbene accumulation suggests that stilbenes have a role in plant defense. Whereas, metabolites accumulated in the newly formed callus tissues presumably have a wider range of roles such as forming new vascular tissues, signalling, defense, and developing a functional graft union. We hypothesize that having additional knowledge of tissue/cell specific gene expression responses to grafting will aid us to understand the potential role of different genes in graft union formation. This experiment would be the first genome-wide single-cell gene expression study on graft union development.

OBJECTIVES: The objective is to characterize the single-cell/tissue transcriptome responses to grafting in grapevine.

METHODS:

Task 1: Optimization of protoplast preparation

The first task will be to optimize protocols to make protoplasts from grapevine callus tissues at the graft interface, ideally we would like to do a time course of gene expression so we need to optimize protoplast preparation at different stages of graft union development, such as, one, two and three months after grafting.

Task 2: Library preparation and data analysis

This task will be done in collaboration with Nathalie Gonzalez and Pascal Martin, Biologie du Fruit et Pathologie, INRAE Bordeaux, France.

Task 3: Tissue-specific gene expression patterns

To complement the single-cell gene expression analysis of the callus, we will also dissect the woody tissues at the graft interface for gene expression analysis (RNAseq or qPCR analysis if the delay in receiving the RNAseq data is too long).

PREREQUISITES:

Interest in molecular biology

REFERENCES :

Feng M, Augstein F, Kareem A, Melnyk CW. 2024. Plant grafting: Molecular mechanisms and applications. Molecular Plant 17, 75-91.

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KEYWORDS (5) : Grapevine, wounding, grafting, RNAseq, transcriptome

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