A fluorescence-based framework to detect local and systemic immune-associated responses in cucumber after chitin oligosaccharide elicitation

Background & Rationale

Plants perceive chitin and related oligosaccharides as conserved microbe-associated molecular patterns, activating pattern-triggered immunity, characterized by reactive oxygen species (ROS), phenylpropanoid activation, and cell-wall reinforcement. While the molecular basis of local immune responses is increasingly well characterized, the systemic propagation of immune signals remains incompletely understood.

Phenylpropanoid-derived compounds, including flavonoids, hydroxycinnamates, and lignin precursors, are intrinsically fluorescent and can substantially affect plant tissue autofluorescence. For this reason, fluorescence spectroscopy represents a promising non-destructive and high-throughput approach to monitor defence-associated metabolic changes in planta This enables fluorescence spectroscopy to serve as a non-destructive, high-throughput proxy for metabolic remodelling associated with plant immune responses. In particular, fluorescence-based analyses may provide an indirect but sensitive proxy for immune-related phenylpropanoid accumulation and cell-wall remodelling.

Objective:

To determine whether leaf local chitin elicitation induces local and **systemic changes in phenylpropanoid metabolism and cell-wall composition** that can be detected and quantified through **autofluorescence spectroscopy**, **correlating with biochemical parameters**.

Cucumis sativus L., is a suitable model for this purpose because it shows I) well-documented systemic immune signaling via phloem-mobile metabolites; II) a strong phenylpropanoid and defence responses; III) accessible phloem-enriched exudate collection. These features make cucumber an attractive experimental system in which to investigate whether local elicitation with chitin oligosaccharides can induce measurable fluorescence changes not only at the site of treatment, but also in distal, untreated leaves. Plants will be grown under controlled conditions, guaranteeing uniform developmental stage to allow to reach the following aims:

Aim 1: Define local and systemic autofluorescence signature of chitin elicitation

- Quantify fluorescence spectral changes in treated and distal leaves.
- Identify spectral markers of immune activation.

Aim 2: Experimentally validate the biological relevance of the fluorescence fingerprint using targeted defence-related molecular and biochemical markers.

- Quantify total phenolics and flavonoids
- Quantify wall-associated phenolics/lignin

Experimental Design

- Treatments: local application of chitin oligosaccharides (primary elicitor); and relative controls.
- Tissues to be sampled: treated leaf (local), untreated distal leaves (systemic), phloem-enriched exudate. Early; intermediate, and late exposures will be sampled.

Methods and approaches that will be used for each aim of the project:

Aim 1: Define local and systemic autofluorescence signatures of chitin elicitation

- Fluorescence spectroscopy
- Fluorescence microscopy (Imaging of fresh tissues for the localization of autofluorescence in the epidermis, vascular tissue, and cell walls)
- ROS detection (by fluorescence microscopy and probes)
- Extraction-based fluorescence (spectophotometer) of soluble phenolics and wall-enriched fractions

Aim 2: Experimentally validate the biological relevance of the fluorescence fingerprint using targeted defence-related molecular and biochemical markers.

- Total phenolics quantification (Folin–Ciocalteu)
- Total phenolics and flavonoids ($AlCl_3$ assay)
- Quantification of wall-associated phenolics/lignin staining or fractionation

Data Analysis, including spectral preprocessing and normalization, multivariate analysis, correlation analysis (e.g. fluorescence vs biochemical markers).