Verticillium wilt on flax: deciphering the tolerance mechanisms through ‘-omics’ approaches

Sylvain LECOMTE
PhD student
FLAX: *Linum usitatissimum*

An ancient crop grown since the beginning of civilization

Two distinct types of flax are cultivated
- Fibers production
- Seeds production
The high quality of the French flax

France is the world’s leader in flax production
- Represents more than 75% of worldwide production
- 95,000 ha
VERTICILLIUM WILT

Major plant disease

Worldwide repartition

Over 400 plant species

Soil-borne plant pathogens

✦ *Verticillium albo-atrum*

✦ *Verticillium longisporum*

✦ *Verticillium dahliae*
**Verticillium wilt of flax**

A misunderstood disease with increasing economic consequences

**Concerning flax,**

- *Verticillium dahliae*
- A major pathogen
- Frequency is increasing
- Significant economic loss

**Complex disease**

- Heterogeneity in the field
- Difficult disease management
- No specific symptoms before harvesting
- Early infection

*From ARVALIS – Institut du végétal*
**Verticillium Wilt of Flax**

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Plants are pulled up

Retting

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VERTICILLIUM WILT OF FLAX

A misunderstood disease with increasing economic consequences

Concerning flax,
- *Verticillium dahliae*
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Complex disease
- Heterogenity in the field
- Difficult disease management
- No specific symptoms before harvesting
- Early infection
- Characteristic symptoms during the retting

Fiber degradation
Verticillium Wilt of Flax

A misunderstood disease with increasing economic consequences

Concerning flax,

✧ *Verticillium dahliae*
✧ A major pathogen
✧ Frequency is increasing
✧ Significant economic loss

Complex disease

✧ Heterogeneity in the field
✧ Difficult disease management
✧ No specific symptoms before harvesting
✧ Early infection
✧ Characteristic symptoms during the retting

Major breeding aim for flax

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Understanding the mechanisms involved in flax defence against *Verticillium dahliae*

Identifying transcriptomic and metabolomic characteristics in order to develop markers for markers-assisted breeding
OBJECTIVES & STRATEGY

Phenotypic characterisation of Verticillium wilt of flax
- Apparition of the necrotic part
- Impact of biomass production
- Quantification of fungi \textit{in planta}

Responses of plants to inoculation
- Transcriptome analysis
- Perturbations in the metabolism

Better understanding of the disease

Improving flax breeding
**PHENOTYPE AND DISEASE CHARACTERISATION**

Assessment of plant reaction to inoculation

**Strong effects of artificial infection** (compared to behaviour in field experiments)

- Symptoms appear between 25-30 DPI
- Chlorosis
- Necrosis
- Premature senescence
- Reduced growth
**Phenotype and Disease Characterisation**

Assessment of plant reaction to inoculation

**Phenotyping**

✧ Disease apparition

![Graph showing apperance of relative necrotic part over days post infection]

**Apparition of relative necrotic part**

- **Early signs of infection on the phenotype**
- **Significant differences between the tolerant cultivar and the susceptible one**

*Student’s t-test*
**PHENOTYPE AND DISEASE CHARACTERISATION**

Assessment of plant reaction to inoculation

**Phenotyping**
- Disease apparition
- Impact on biomass

### Impact of *V. dahliae* on biomass

![Graph showing impact of *V. dahliae* on biomass](image)

- **Significant impact on biomass production**
- **Particular behaviour 20 DPI**
**PHENOTYPE AND DISEASE CHARACTERISATION**

Assessment of plant reaction to inoculation

**Phenotyping**
- Disease apparition
- Impact on biomass
- Fungi quantification

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**Quantification of *V. dahliae* in flax**

Days post infection vs. DNA ratio of *V. dahliae* to Flax DNA.

- **V. dahliae** levels are higher in the susceptible cultivar.
- Tolerant cultivar is able to maintain a lower amount of fungi within its tissues.

*Student’s t-test*
TRANSCRIPTOMICS

Microarrays - genes expression in response to infection 7 DPI

✧ Tolerant cultivar: infected VS control

- 39 differentially expressed genes
- 15 genes involved in plant response to stress

✧ Susceptible cultivar: infected VS control

- 9 differentially expressed genes
- 2 genes involved in plant response to stress

Little impact on transcriptome

Host transcriptional responses to infection are different 7 DPI

Benjamini and Hochberg correction
TRANSCRIPTOMICS
Microarrays – comparison between the two models 7 DPI

✧ Controlled conditions: tolerant VS susceptible

✧ Infected conditions: tolerant VS susceptible

552 differentially expressed genes

Constitutive tolerance?

456 differentially expressed genes

The cultivar differences are more important than the transcriptional responses to the artificial infection 7 DPI

Tolerance predispositions?
Transcript abundance levels of defence-related genes are higher in the tolerant cultivar

Benjamini and Hochberg correction
**TRANSCRIPTOMICS**

RT-qPCR : 7 DPI

**PR4**

![PR4 expression graph]

**β-1,3-glucanase**

![β-1,3-glucanase expression graph]

**Chitinase**

![Chitinase expression graph]

**Disease resistance-responsive (dirigent-like)**

![Disease resistance-responsive expression graph]

**MLP like**

![MLP like expression graph]

**Pleiotropic drug resistance**

![Pleiotropic drug resistance expression graph]

**Peroxidase**

![Peroxidase expression graph]

Differences between cultivars

Repression of genes expression upon infection
**TRANSCRIPTOMICS**

RT-qPCR : 20 DPI

**PR4**

- **β-1,3-glucanase**
- **Chitinase**
- **Disease resistance-responsive (dirigent-like)**
- **MLP like**
- **Pleiotropic drug resistance**
- **Peroxidase**

**Differences between cultivars**

*Genes of the tolerant cultivar are up-regulated upon the infection*
METABOLOMICS

NMR: multivariate analysis

Separation between harvest time

Separation between conditions

The pathogen affects the metabolism
METABOLIC PATHWAYS

**Glycolysis**

- Pyruvate

**Krebs cycle**

- Malate
- Fumarate
- Succinate
- α-ketoglutarate

**Spermine biosynthesis pathway**

- Glutamate
- Glutamine
- Proline
- Arginine
- G-aminobutyric acid

**Molecular Biomarkers**

- Spermine
- Putrescine

NMR: targeted analysis
Metabolomics

NMR: targeted analysis

Metabolites:
- Glycolysis
- Pyruvate
- Shikimate
- Krebs cycle
- Flavonoids
- Phenylpropanoids
- Tryptophane
- Phenylalanine
- Tyrosine
- Cafeic acid
- Ferulic acid
- Coumaric acid

Metabolites:
- Phospholipid
- Lipid
- Carbohydrate
- Nucleic acid
- Protein
- Amino acid
- Fatty acid
- Ketone
- Alcohol
- Esters
- Aldehydes
- Organic acids
CONCLUSION AND PERSPECTIVES

Phenotypic characterisation of Verticillium wilt of flax

Characterisation of the disease
Field experiments
Artificial infection in greenhouses

Breeding

Assessment of plant reaction to artificial inoculation

Significant differences between the tolerant and the susceptible cultivars
Field experiments
CONCLUSION AND PERSPECTIVES

Phenotypic characterisation of Verticillium wilt of flax

Characterisation of the disease

- Field experiments
- Artificial infection in greenhouses

Breeding

Other notations
CONCLUSION AND PERSPECTIVES

Transcriptome analysis of flax inoculated by *V. dahliae*

Characterisation of the disease

Field experiments

Artificial infection in greenhouses

Transcriptomics

Breeding

Microarrays

RT-qPCR

Two cultivars
Two different responses to the pathogen

Tolerance predisposition?

Differences in the responses involved in flax response against *Verticillium dahliae*
CONCLUSION AND PERSPECTIVES

Transcriptome analysis of flax inoculated by *V. dahliae*

- **Characterisation of the disease**
  - Field experiments
  - Artificial infection in greenhouses

- **Transcriptomomics**
  - Microarrays
  - RT-qPCR

Study expression pattern of other genes involved in defence mechanisms

Study the response to *V. dahliae* 20DPI with another microarrays experiment
CONCLUSION AND PERSPECTIVES

Perturbations in the metabolism of flax infected with *V. dahliae*

**Characterisation of the disease**

- Field experiments
- Artificial infection in greenhouses

**Breeding**

- Transcriptomics
  - Microarrays
  - RT-qPCR
- Metabolomics
  - NMR

Significant impact of infection on the metabolism

Link between some genes and pathways
CONCLUSION AND PERSPECTIVES

Perturbations in the metabolism of flax infected with *V. dahliae*

Characterisation of the disease
- Field experiments
- Breeding
  - Microarrays
  - RT-qPCR
- Artificial infection in greenhouses
  - Transcriptomics
  - Metabolomics
    - NMR
  - LC/MS

Identification of metabolites that are responsible for the classification
**CONCLUSION AND PERSPECTIVES**

Perturbations in the metabolism of flax infected with *V. dahliae*

- **Field experiments**
- **Artificial infection in greenhouses**

**Characterisation of the disease**

- **Breeding**
- **Transcriptomics**
  - Microarrays
  - RT-qPCR
- **Metabolomics**
  - NMR

**Link between the two approaches**
CONCLUSION AND PERSPECTIVES

Mechanisms involved in flax defence against *V. dahliae*

- Characterisation of the disease
  - Field experiments
  - Artificial infection in greenhouses
    - Transcriptomics
      - Microarrays
      - RT-qPCR
    - Metabolomics
      - NMR

Better understanding of flax responses to *V. dahliae*
Comprehension of the disease and tools for molecular breeding
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