

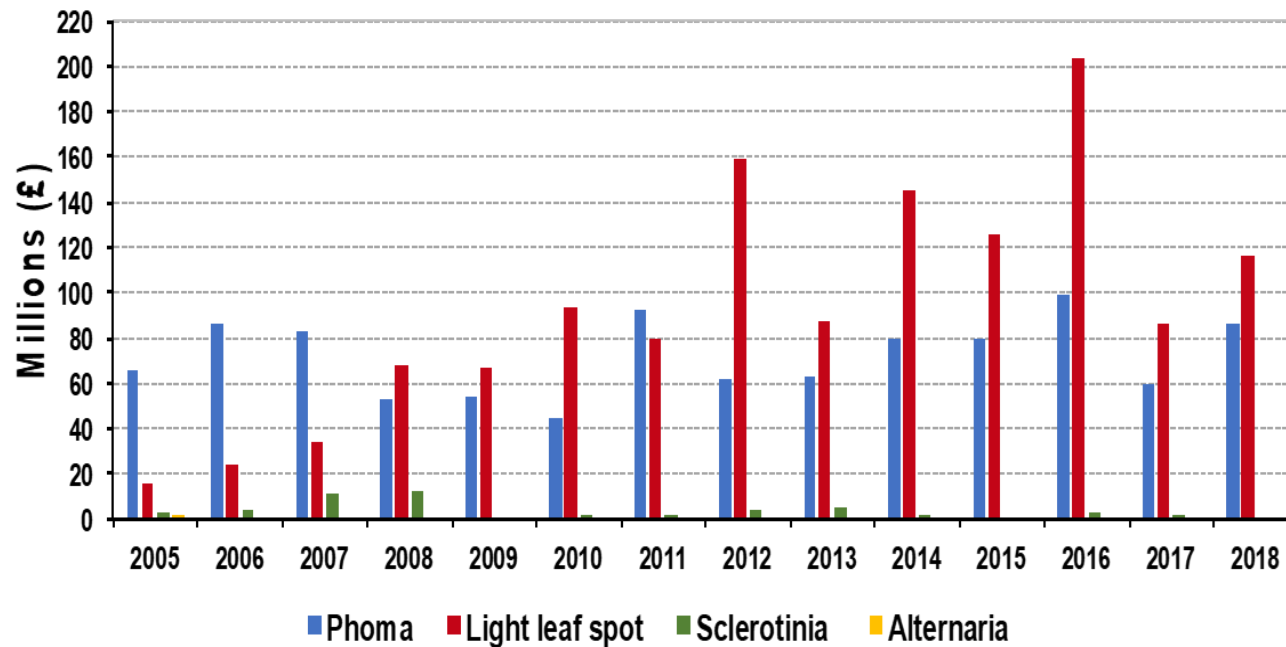
Laura Gimenez Molina

Characterization of durable quantitative  
resistance of *Brassica napus* (oilseed rape)  
against the light leaf spot pathogen  
*Pyrenopeziza brassicae*

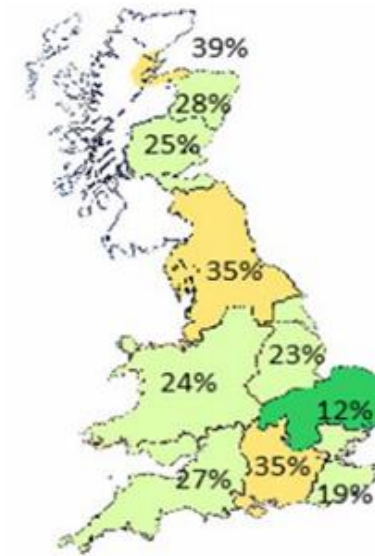
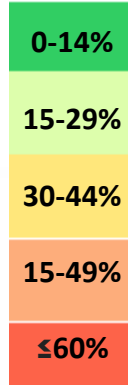
# Impact of light leaf spot (LLS)

- **Oilseed rape** (*Brassica napus*) is the most important temperate oilseed crop in the world, however it is severely affected by the **light leaf spot disease**.
- Oilseed rape annual yield loss due to LLS > **£100 M**

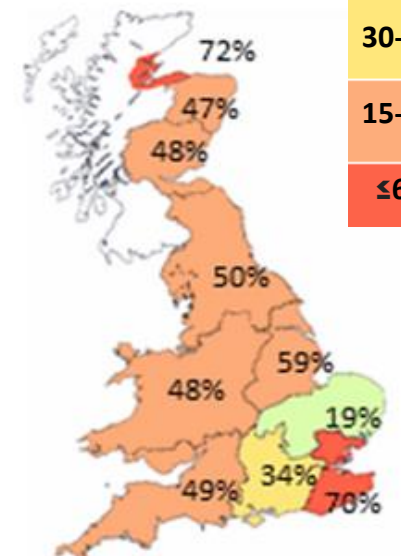
Winter oilseed rape yield losses caused by different diseases (England & Wales)



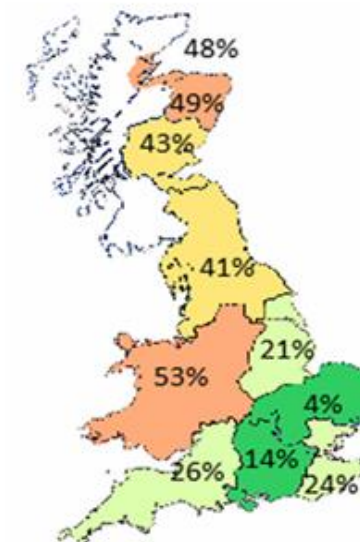
Percentage of crops with >25% affected plants (England & Wales, AHDB)



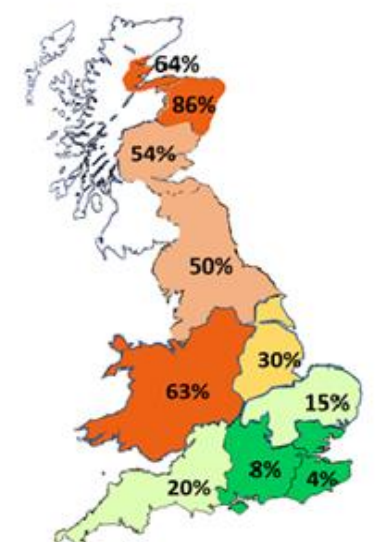
2013/14



2016/17



2018/19



2020/21

# Pyrenopeziza brassicae life cycle

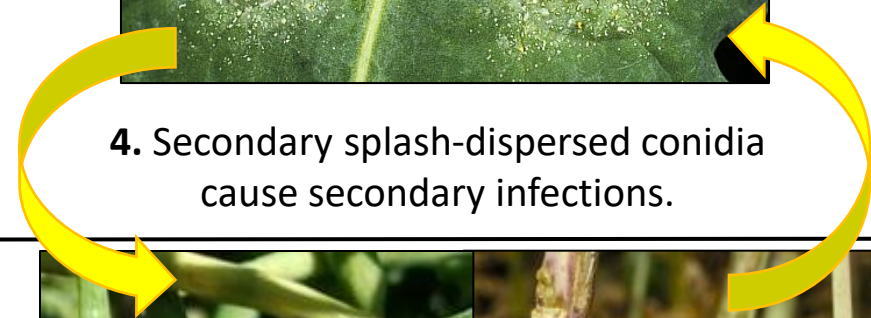


**2.** Ascospores germinate and penetrate the cuticle. Hyphae growth in the sub-cuticular space.  
(Boys *et al.* 2012)



**3.** Conidial production and asexual sporulation.

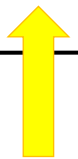
**4.** Secondary splash-dispersed conidia cause secondary infections.



**1.** Primary infection by wind-dispersed ascospores.

**AUTUMN**

**WINTER**



**7.** Production of ascospores.



**SUMMER**

**SPRING**

**5.** Infection of leaves, stems, meristems and pods, causing lesions.



**6.** Development of apothecia on infected debris.

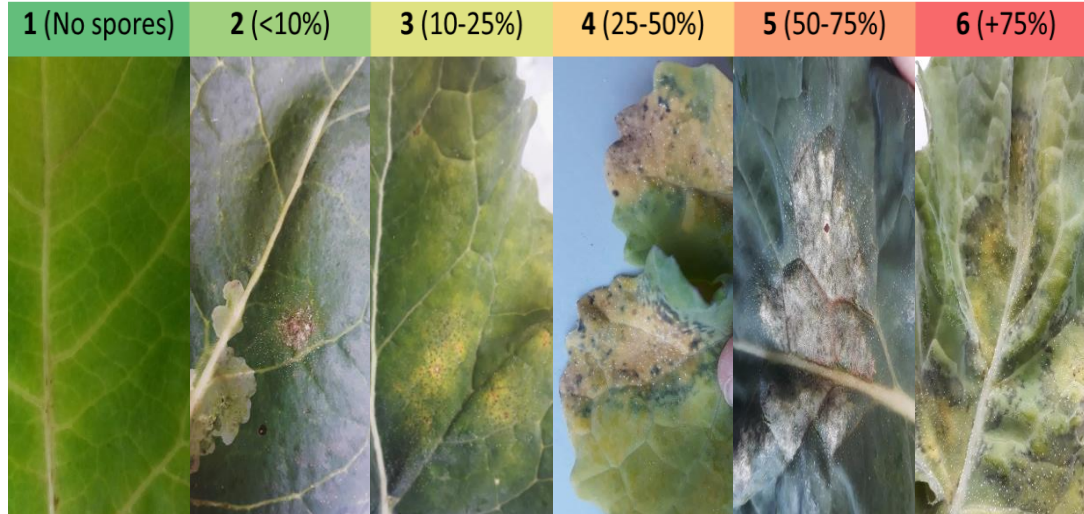


# Aims and Objectives

- **Chapter 1:** Improvement of phenotyping strategies
  - Spore counting
  - Digital imaging
  - WAC assay
  - qPCR
- **Chapter 2:** Characterisation of the function of candidate genes
  - RNAseq analysis
  - TILLING mutants
  - Study of gene expression of resistant and susceptible lines
- **Chapter 3:** Identification of QTLs for resistance against *P. brassicae*
  - Biparental cross
  - Bulked segregant analysis of the F<sub>2</sub> population

# Chapter 1: Improvement of phenotyping strategies

## Current method: visual assessment



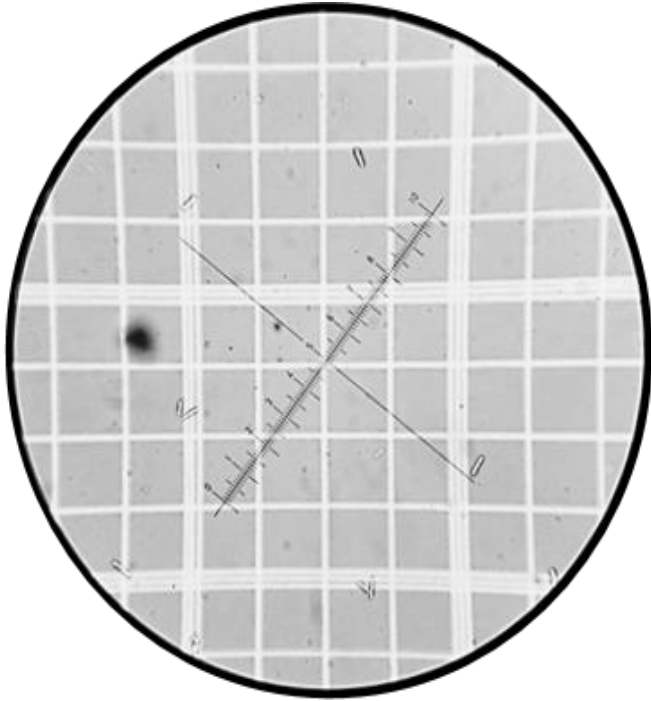
Score	Description
1	No sporulation
2	<10% leaf area with sporulation
3	10-25% leaf area with sporulation
4	25-50% leaf area with sporulation
5	50-75% leaf area with sporulation
6	75-100% leaf area with sporulation

## Quantitative methods:

- Spore counting
- Digital imaging
- Wheat-germ agglutinin chitin (WAC) assay
- qPCR

# Chapter 1: Improvement of phenotyping strategies

**Quantitative methods:** Spore counting



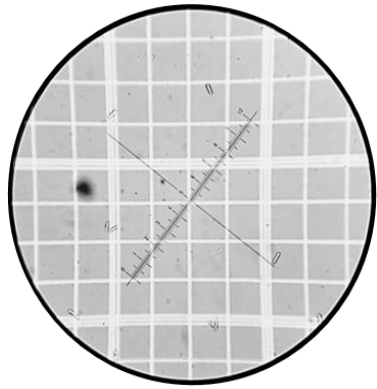
$10^4$  spores/ml  
Aurelia



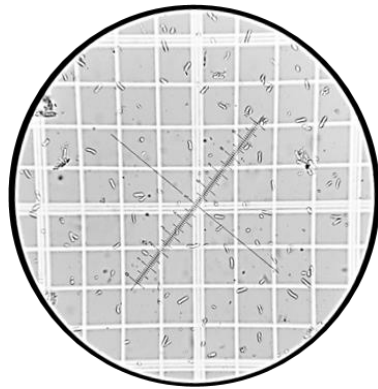
$10^6$  spores/ml  
Leopard

# Chapter 1: Improvement of phenotyping strategies

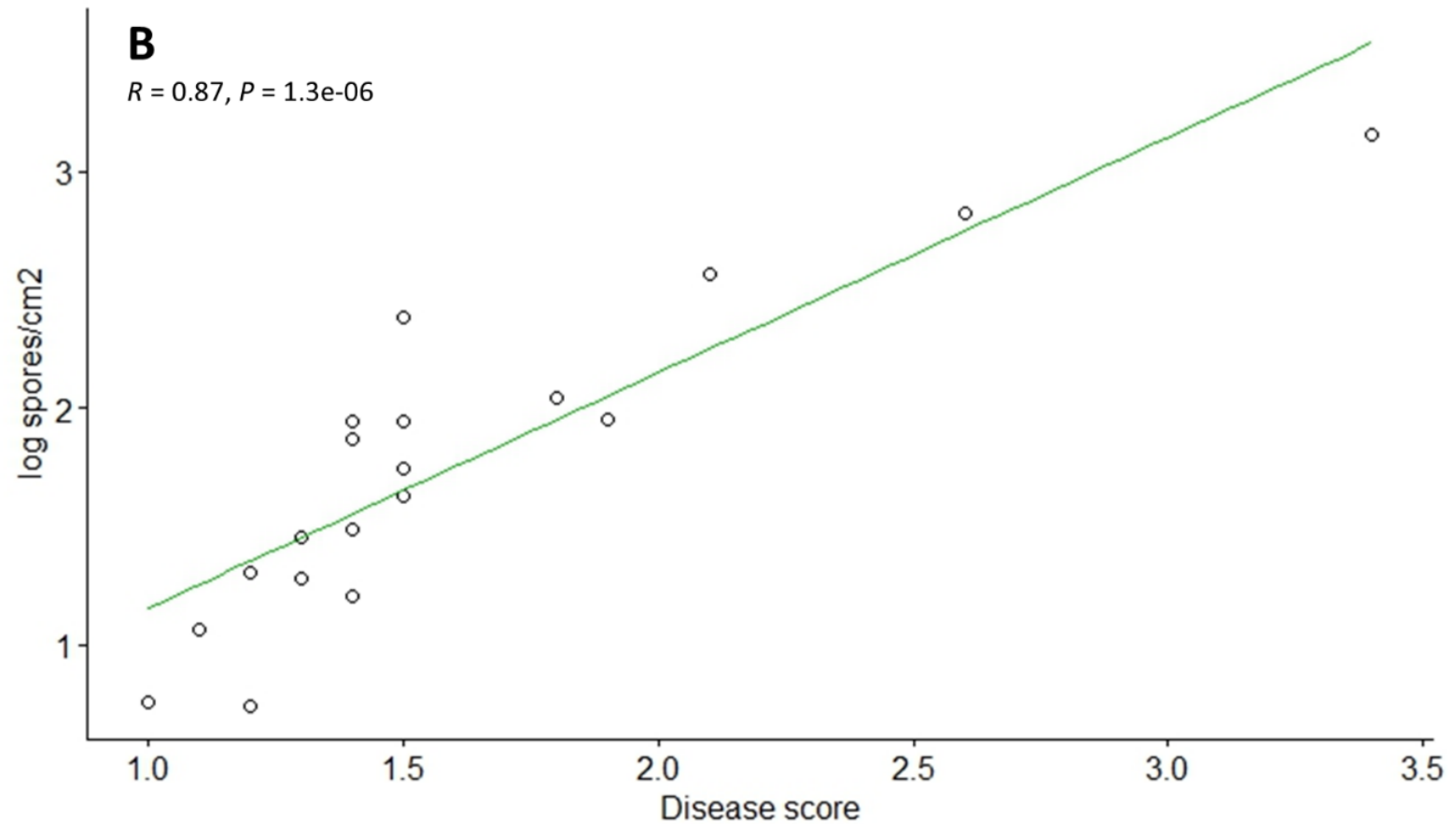
## Quantitative methods: Spore counting



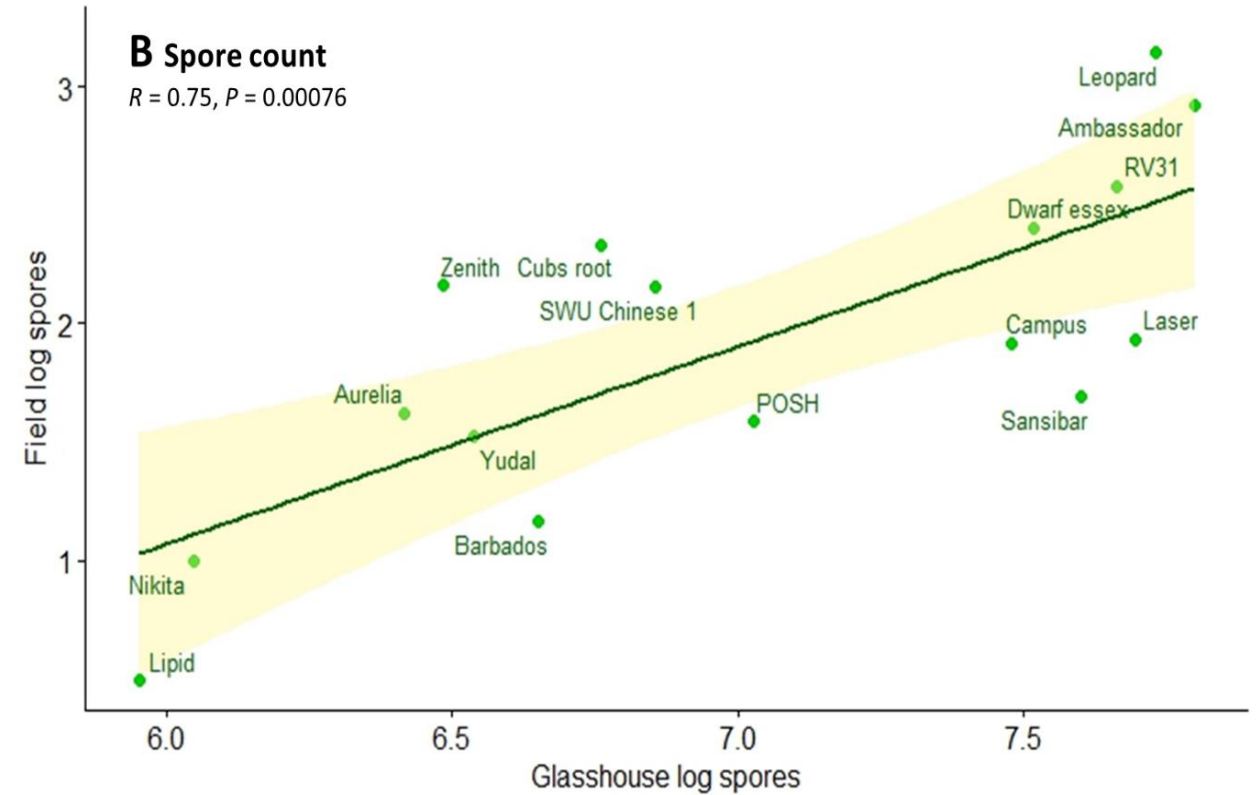
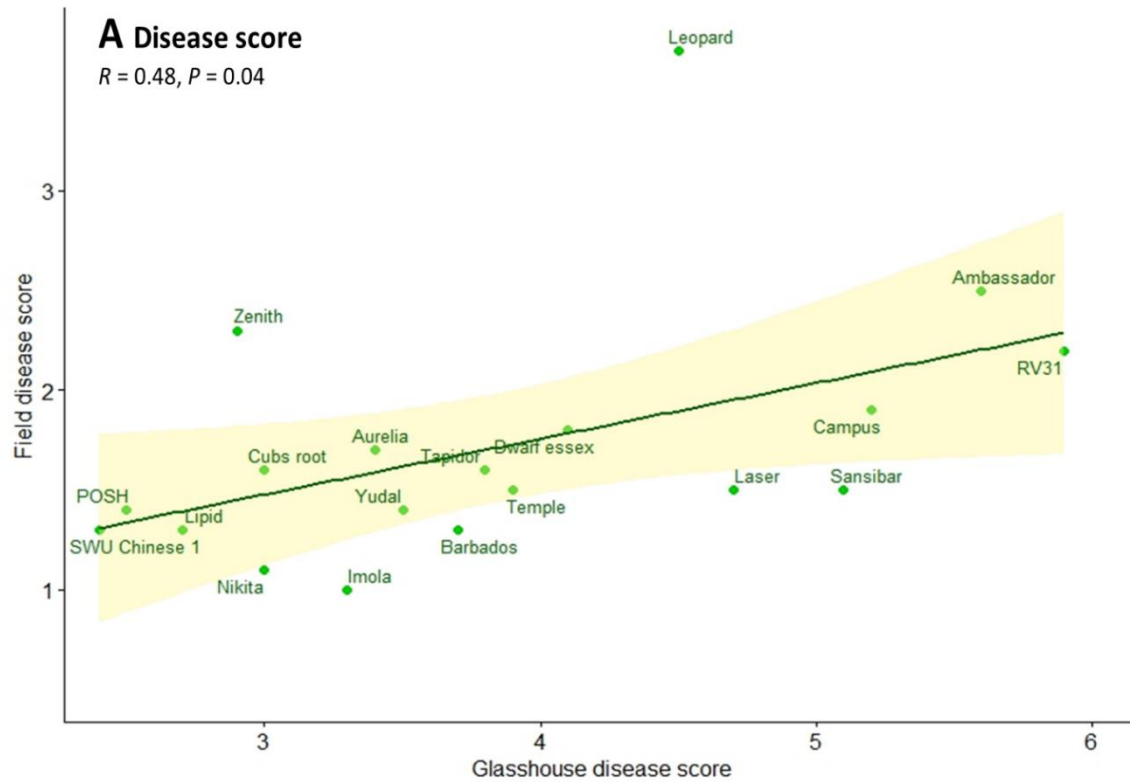
$10^4$  spores/ml  
Aurelia



$10^6$  spores/ml  
Leopard



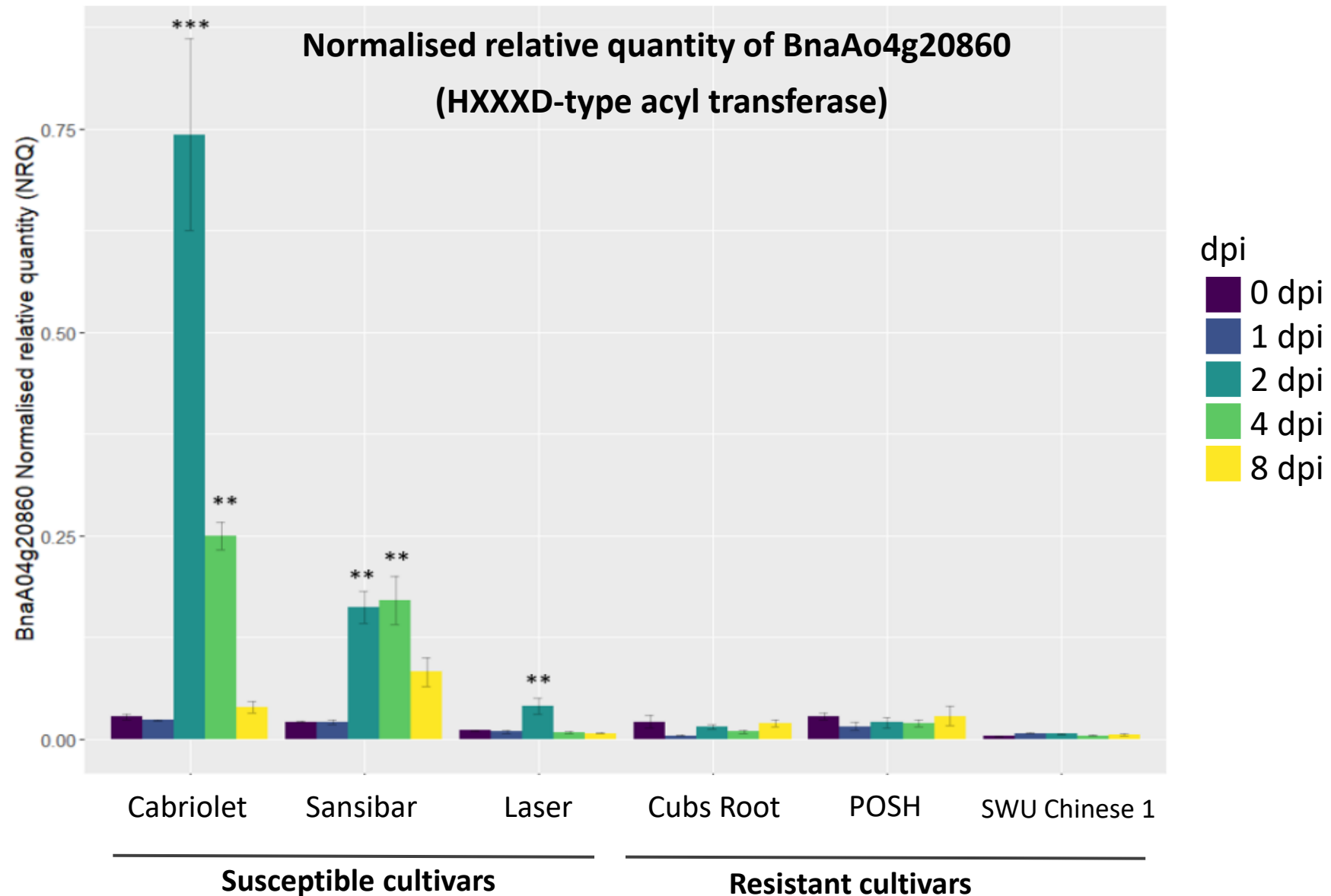
# Chapter 1: Improvement of phenotyping strategies



Scatter plots of the different methods used to phenotype the severity of the disease:  
**A) visual assessment and B) spore counting.**

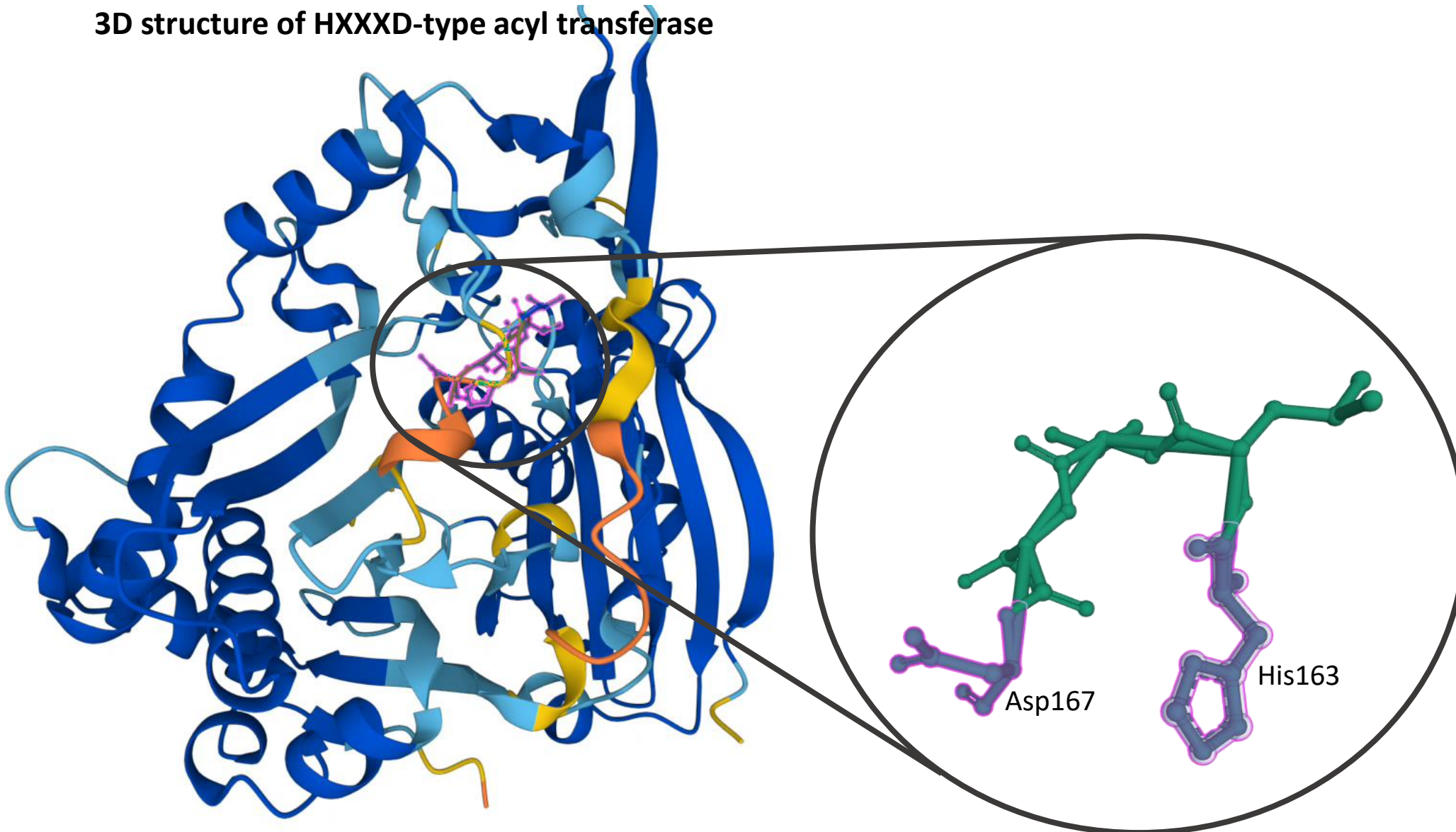


# Chapter 2: Characterisation of the function of candidate genes



# Chapter 2: Characterisation of the function of candidate genes

3D structure of HXXXD-type acyl transferase



# Chapter 2: Characterisation of the function of candidate genes

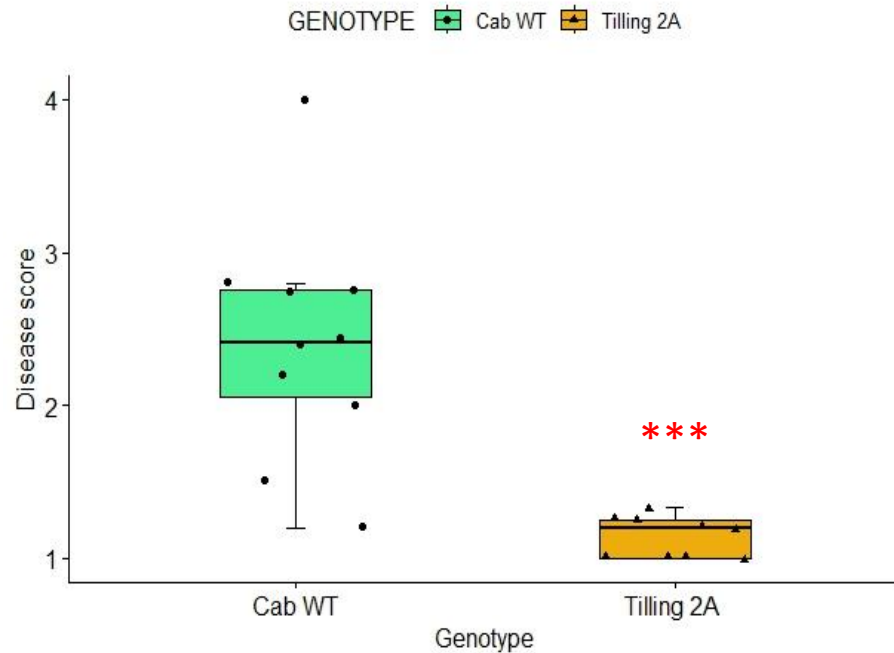
## TILLING mutants

TILLING mutant	Biological replicate	Sequence of WT														Mutation Change	Het/Hom	Missense	Truncations	
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7		G/A or C/T	Coding Region	Stop
2	A	C	G	T	T	A	C	A	G	A	C	G	G	A	G	C	G>A	Mutant Hom	499G>A:167D>N (Aspartate to Asparagine)	
2	C	C	G	T	T	A	C	A	G	A	C	G	G	A	G	C	G>A	Het	499G>A:167D>N (Aspartate to Asparagine)	
2	D	C	G	T	T	A	C	A	G	A	C	G	G	A	G	C	G>A	Het	499G>A:167D>N (Aspartate to Asparagine)	
3	A	C	G	G	T	C	T	G	G	A	G	T	T	T	T	T	G>GA	Het		516G>GA:172W>X (Tryptophane to Stop)
3	B	C	G	G	T	C	T	G	G	A	G	T	T	T	T	T	G>GA	Het		516G>GA:172W>X (Tryptophane to Stop)
4	F	A	A	A	C	C	T	G	G	A	C	G	G	A	G	A	G>GA	Het		534G>GA:178W>X (Tryptophane to Stop)
6	A	A	A	G	T	C	T	T	C	T	C	T	T	T	C	A	C>CT	Mutant Hom	191C>CT:64S>S/F (Serine to Phenylalanine)	

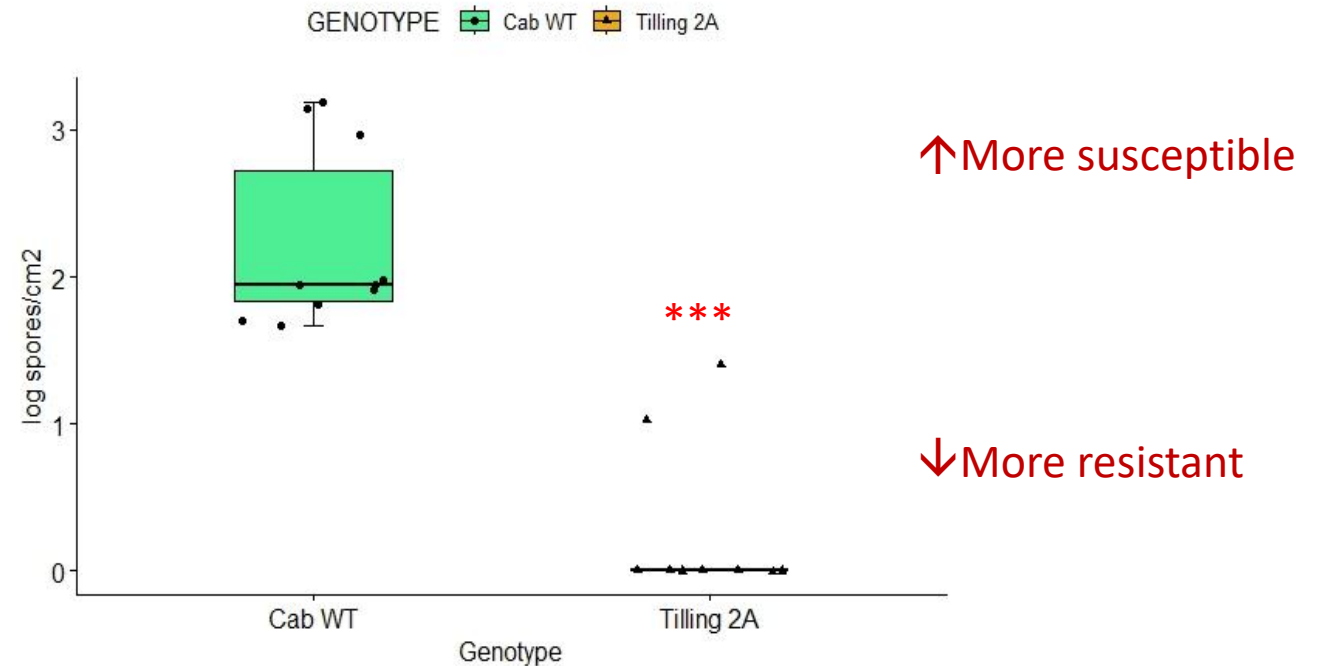
# Chapter 2: Characterisation of the function of candidate genes

## TILLING mutants

### Visual assessment

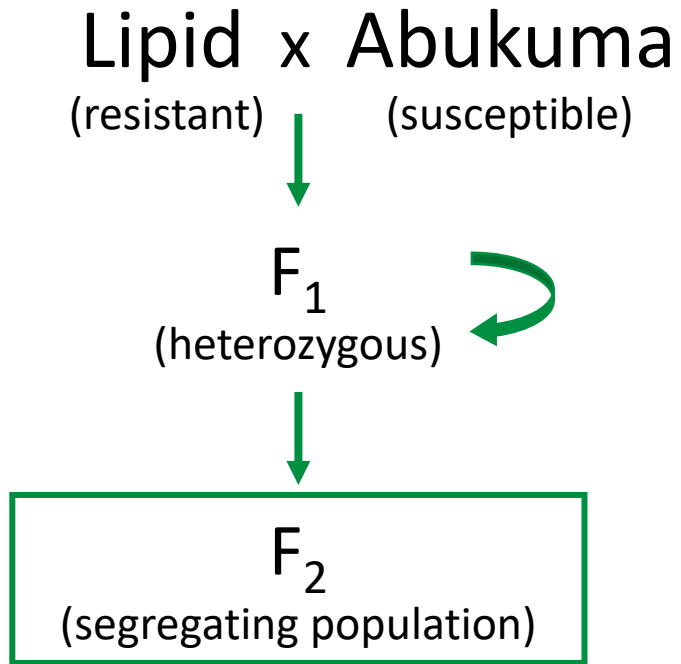


### Spore count

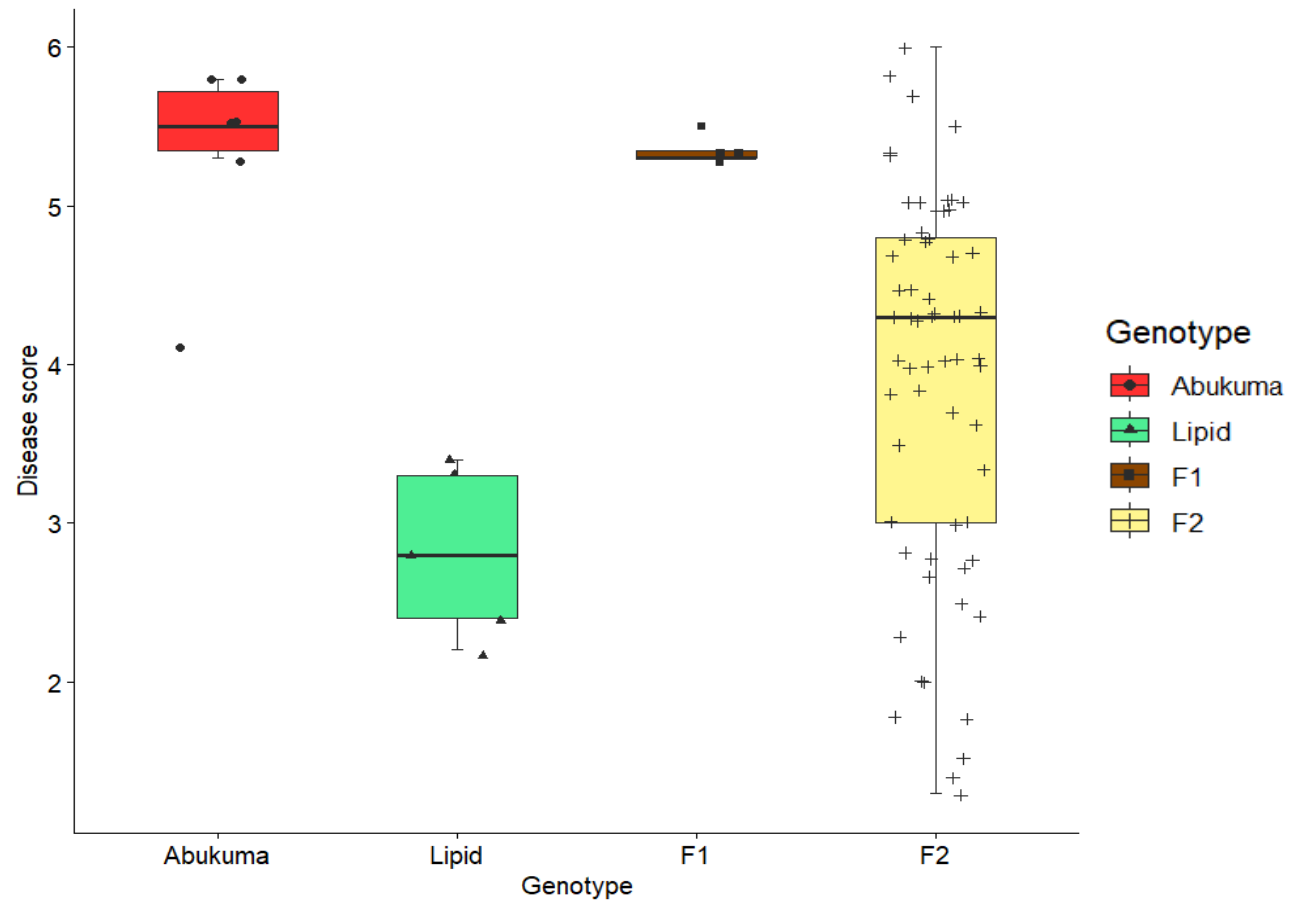


*There is a statistically significant difference ( $P < 0.001$ ) between WT and TILLING mutant 2A using both the visual assessment method ( $P$ -value = 0.0008676) and the spore count method ( $P$ -value = 0.0001945).*

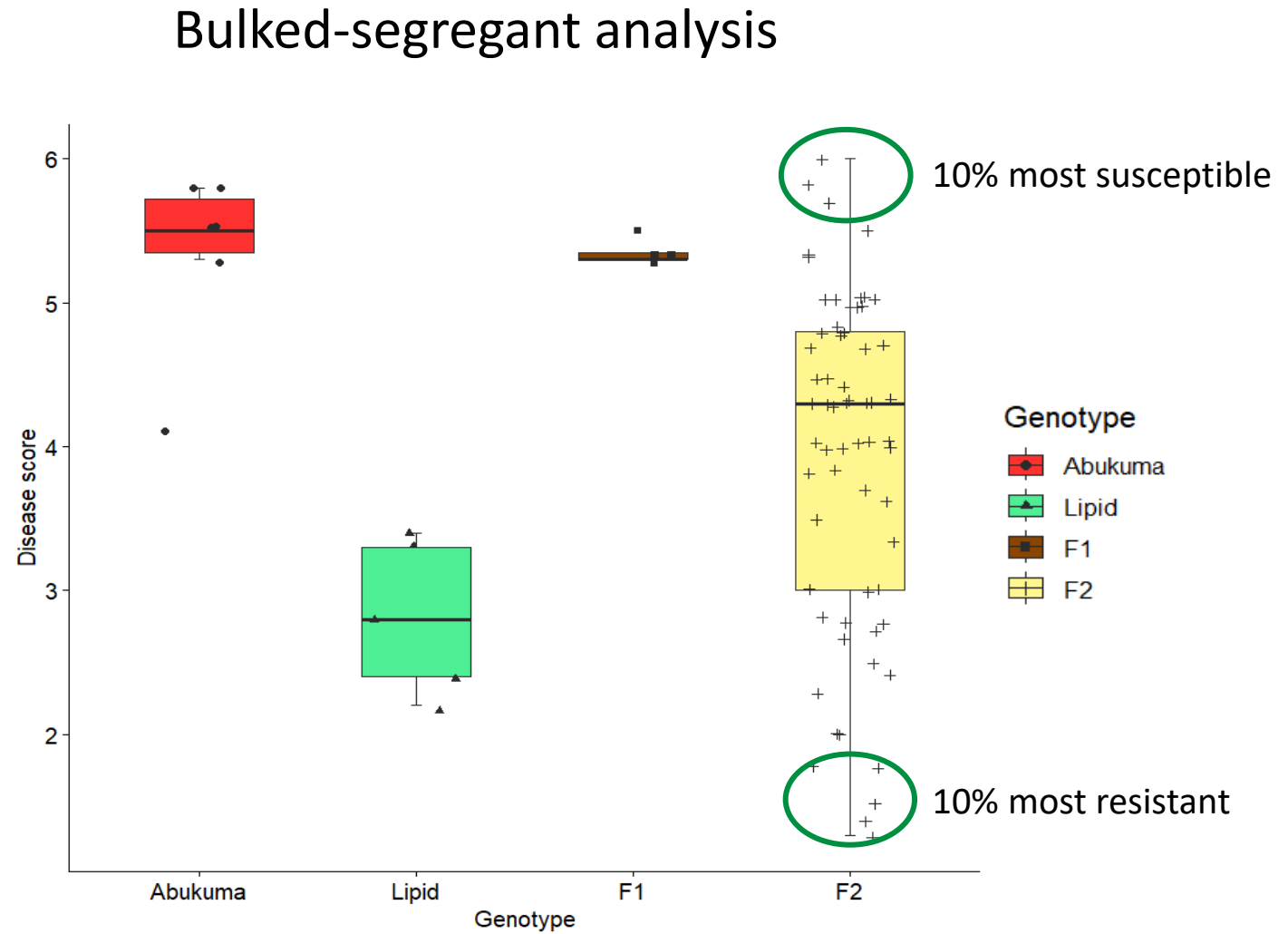
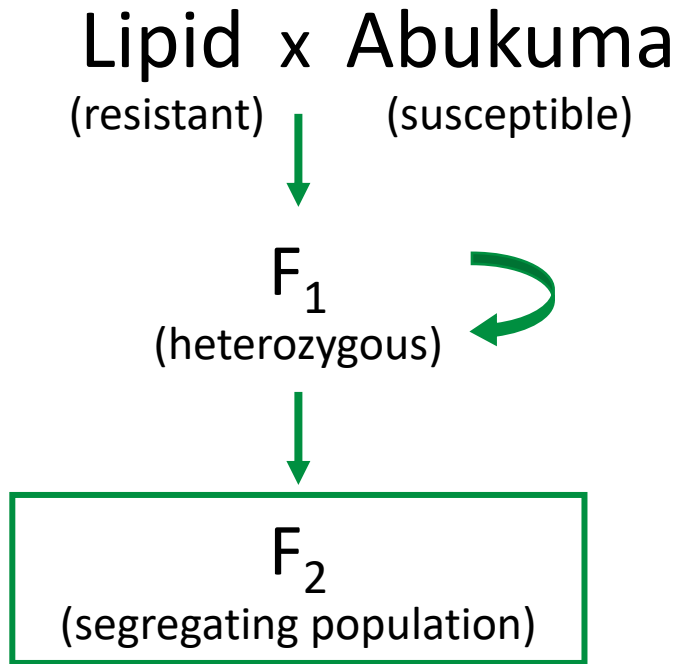
# Chapter 3: Identification of QTLs for resistance against *P. brassicae*



Phenotyping of the F<sub>2</sub> population



# Chapter 3: Identification of QTLs for resistance against *P. brassicae*





# Acknowledgements



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*Unlocking Nature's Diversity*

**John Innes Centre**

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**KWS**



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