
SLU
SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE

Short Scientific Mission (STSM)

Functional genomic of cereals and the opportunity to accelerate the development of resistant cereal varieties and reduce the use of chemical treatments

Host Scientist, Laboratory and Institute: Dr. Dimitar Douchkov, the Biotrophy and Immunity Lab, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Applicant: Vahidehalsdata Rafieebanadaki, Researcher, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Science (SLU), Sweden.
Vahideh.rafiei@slu.se

April 16-28, 2023

Background of my current project: Fusarium head blight (FHB)

- FHB is a devastating cereal disease in Sweden and globally, causing significant reductions in yields and mycotoxin contamination of the grain

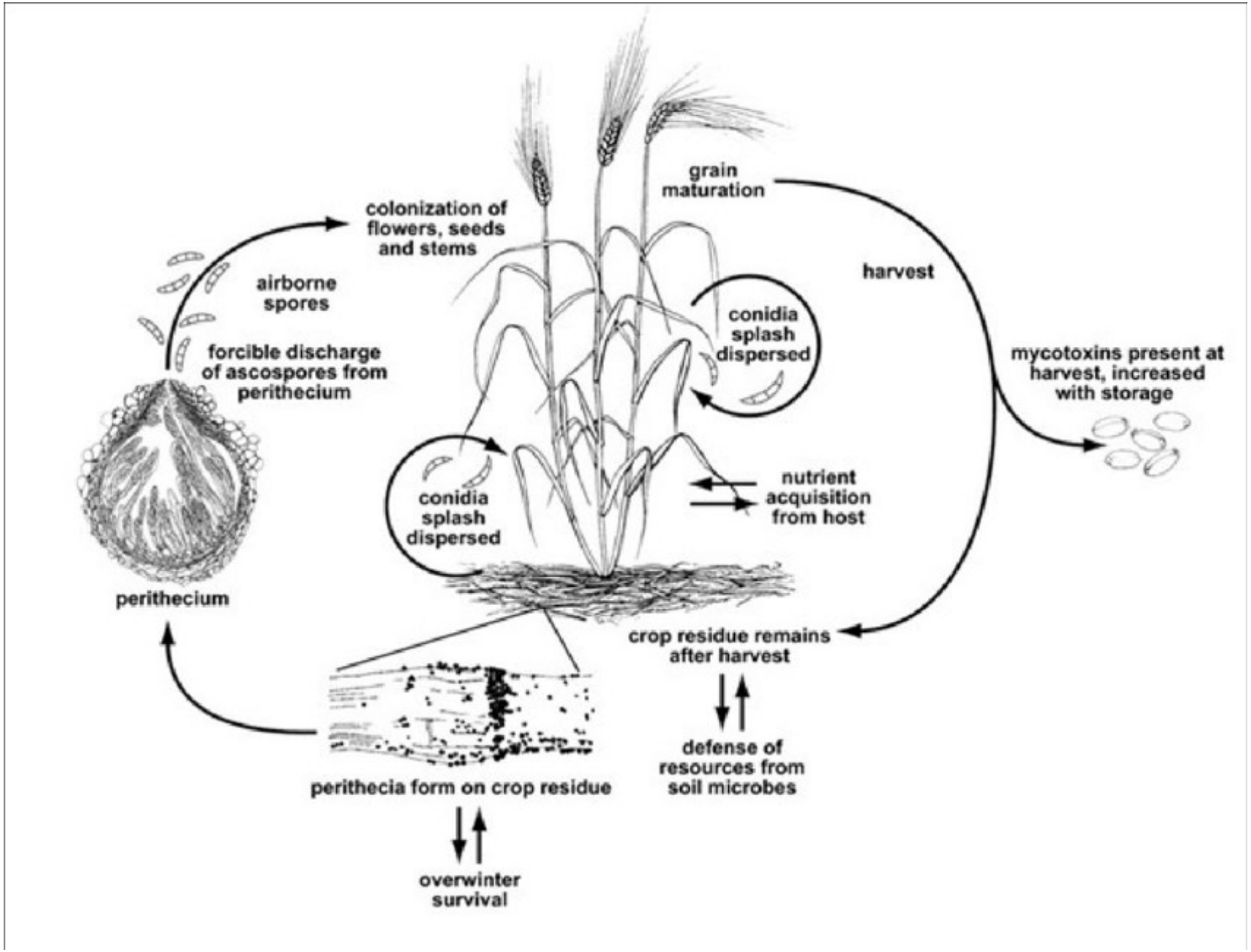
- Several *Fusarium* species can cause FHB

- *F. graminearum* species complex (FGSC) are considered the main cause of FHB epidemics in wheat and barley worldwide

Symptoms of *Fusarium* head blight (FHB)

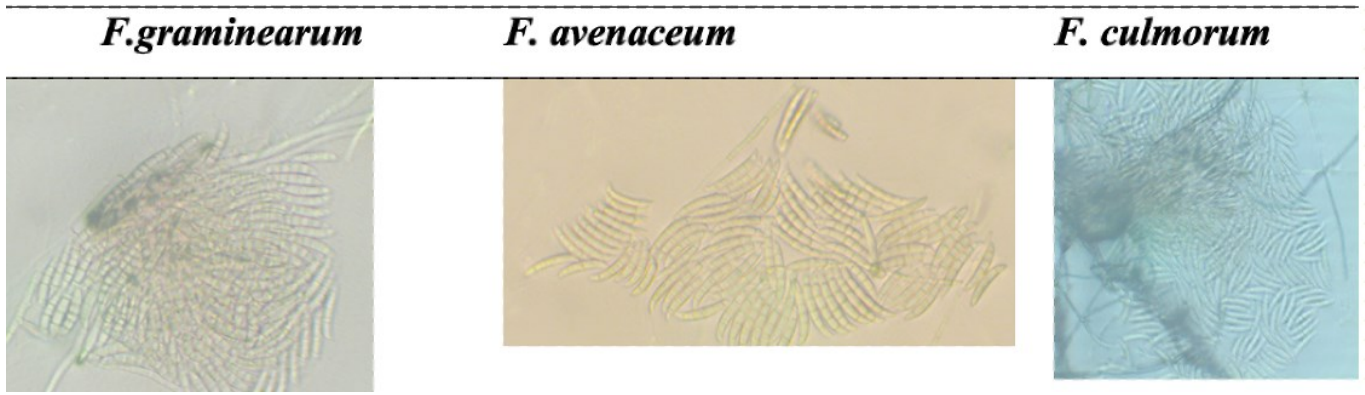
Image: International Maize and Wheat Improvement Center

Symptomatic heads with bleached spikelets. (**b**) Premature bleached head with pinkish-red mycelium and spores on infected spikelets. (**c**) *Fusarium*-damaged grain showing pink and white discolorations.
Birr et al., .*Microorganisms* **2020**, *8*, 617. https://doi.org/10.3390/microorganisms8040617



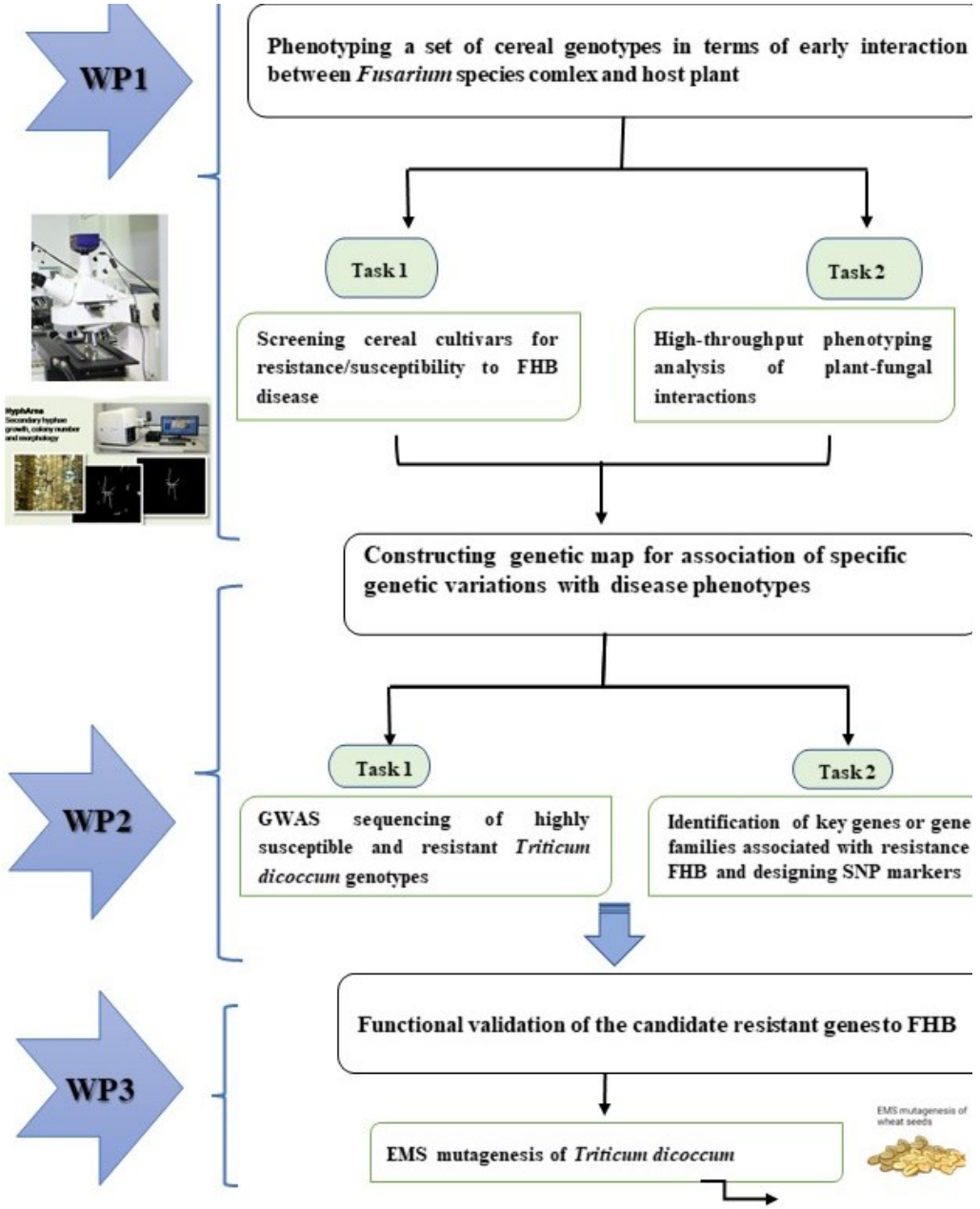
Shude et al., 2020. DOI: 10.17159/sajs.2020/7854

Fusarium species complex assosciated with FHB



- **Over 17 Fusarium species are known to be associated with FHB.**
- ***Fusarium graminearum* species complex the main source of FHB worldwide**

Rafiei et al., 2024, unpublished



Overview of the proposed research project

Objective of the work carried out during the STSM

Host Scientist, Laboratory and Institute: the Biotrophy and Immunity Lab, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

To get comprehensive hands-on training in functional studies of plant-fungal intearction, including:

- The design and execution of gene delivery in cereals via the particle bombardment method.

Currently, there is a gap in functional genomic studies at SLU and we need to further develop knowledge and techniques regarding gene function studies in pathogen-cereal interactions. Therefore, this invaluable opportunity using the support from STSM grant, has equipped me with a deeper understanding of transient gene expression techniques and study of gene function in plant species

Barley powdery mildew disease

- Powdery mildew is one of the most consistently damaging diseases of barley in Europe, caused by the obligate biotrophic fungus ***Blumeria graminis*** **f.sp.** ***hordei***
  - The fungus can infect all green plant parts causing premature tissue senescence leading to severe reductions in yield
- Much effort is devoted to the exploitation of genetic plant resistance for disease control.

- However, most single genes for resistance in barley have proved as virulent pathogen races capable of breaking down the resistance.
- *Mildew Locus O* (MLO) genes in barley have proved to be an exception.
- These genes make plants more susceptible to powdery mildew by helping the fungus penetrate plant cells.
- Currently, this powdery mildew resistance is the most used resistance in spring barley grown throughout Europe.

- Mutations that *disable* MLO genes (*loss-of-function* mutations) have been shown to *increase* durable and broad-spectrum resistance to powdery mildew
  - This approach is widely used to protect crops like barley, wheat, and tomatoes against powdery mildew
- The mlo powdery mildew resistance effectively prevents the mildew fungus from penetrating host epidermal cells, and thus prevents fungal haustorium formation and infection.

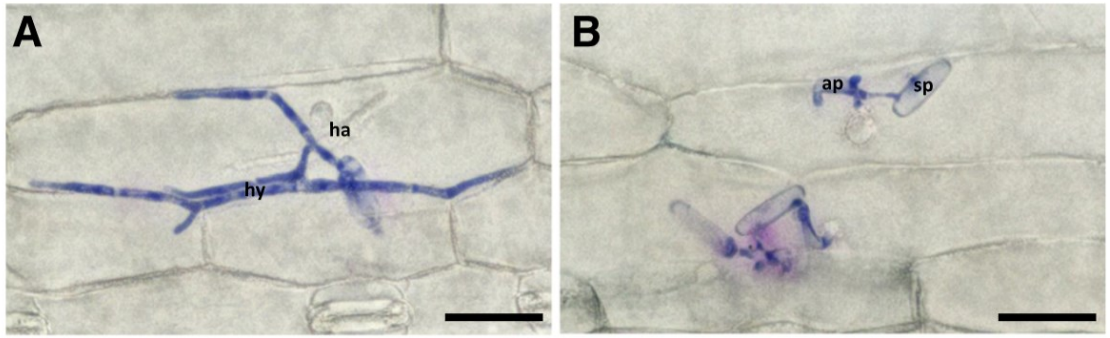
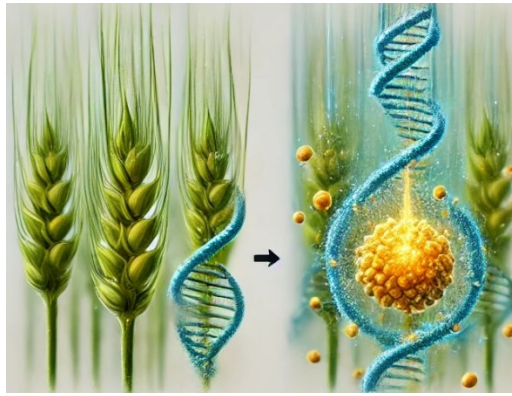


Fig. 1. *mlo*-based resistance prevents host cell penetration. Barley leaves were inoculated with *Blumeria graminis* f. sp. *hordei* (isolate K1) and photographs were taken at 48 h postinoculation. Fungal structures were stained with Coomassie brilliant blue. **A,** The plant lines were the susceptible cultivar Ingrid and **B,** *mlo*-3 in Ingrid background. sp = spore, ap = appressorium, ha = haustorium, and hy = hyphae. Scale bar = 30 µm.

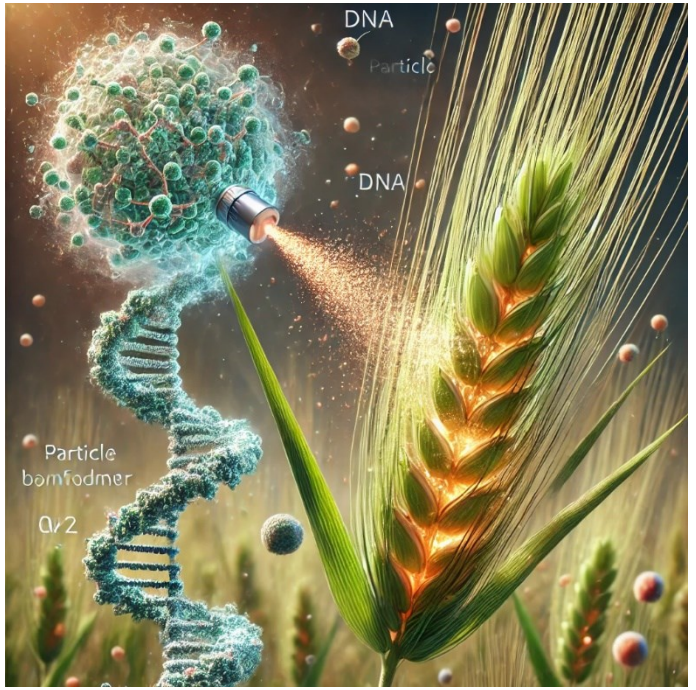
Kusch & Panstruga, 2017, MPMI Vol. 30, No. 3, 2017, pp. 179–189

In barley, *mlo* resistance is of great agronomic importance given that approximately one-half of the current European spring varieties is of the *mlo* genotype and thus highly resistant against *Blumeria graminis* f.sp. *hordei*

Particle bombardment (biolistic gene trasnfer)



- Particle bombardment, is a technique used to introduce genes of interest into plant cells and observe the effects of gene expression in plant.
- A technique to deliver DNA directly into plant cells using high-speed particles (gold)
- It has been widely used in cereal studies for studying gene function and plant disease resistance.



Particle bombardment is a versatile technique and is helpful for plants that don't easily take up DNA through other methods, like *Agrobacterium*-mediated transformation.

The experimental work at IPK

- Transient gene expression of MLO gene, negative regulator of the resistance against *Blumeria graminis* f. sp. *Hordei,* using particle bombardment method

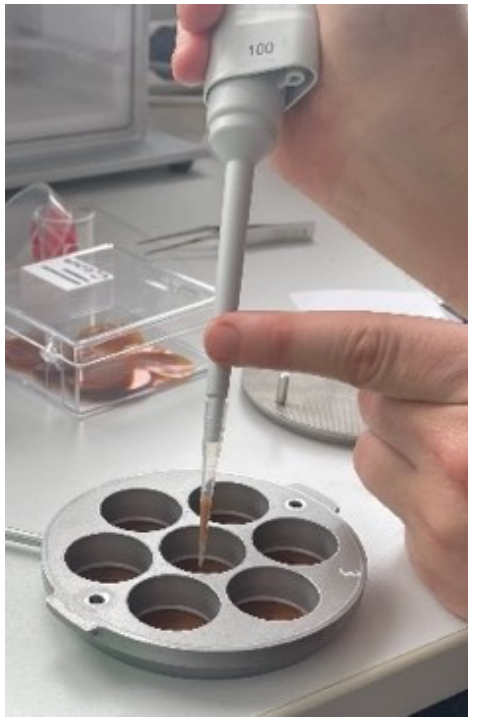
- Plant Cells shot by overexpression and silencing constructs of MLO using the PDS-1000/He Particle Delivery System

- Each replicate of each genotype was shot with different constructs. Two with overexpression constructs which can over express the MLO gene in the plant cells, one construct with only reporter as a control and one construct with silencing construct which could silence the MLO gene.
- The leaves of all genotypes were infected by powdery mildew afterward.

- The resistance changes scored microscopically as a ratio of infected to non-infected transformed cells.
- The transformed cells were visualized with the help of corresponding specific staining.

Preparing the leaf samples of four different barley genotypes for Particle bombardment

Steps for Creating MLO Mutants Using Particle Bombardment



- **Design the Gene-Editing Construct:** Overexpression and silencing constructs of MLO were used
- **Prepare the Particles**: Coat gold particles with the MLO-targeting DNA construct
- **Bombard the Plant Cells**:The coated particles were loaded into the gene gun. Bombard plant tissue to deliver the construct into plant cells.
- **Testing for Powdery Mildew Resistance**: Inoculate the MLO mutant plants with powdery mildew spores.
- Monitor for reduced infection, indicating successful resistance

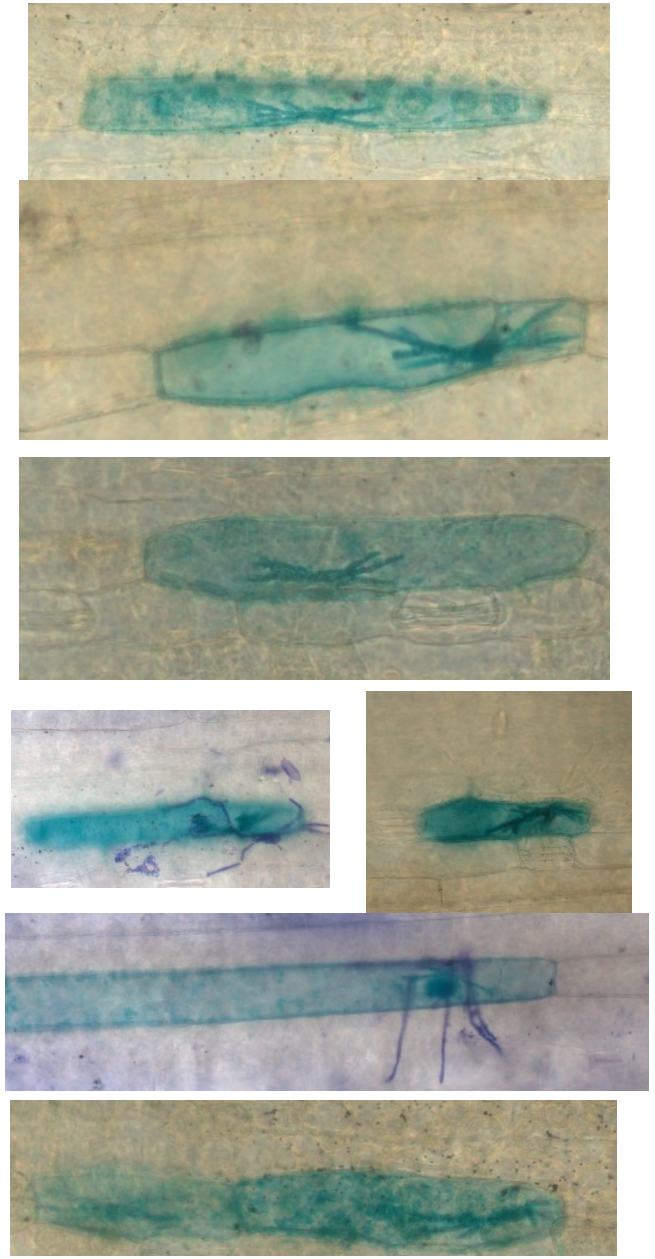
DNA construct for silencing and overexpressing the Mlo gene were transiently transformed into the leaf epidermal cells of different barley genotypes deficient or normally expressing the Mlo gene using Particle bombardment and shooting with the PDS-1000/He Particle Delivery System available at IPK.

Results of experimental work during practical visit at IPK

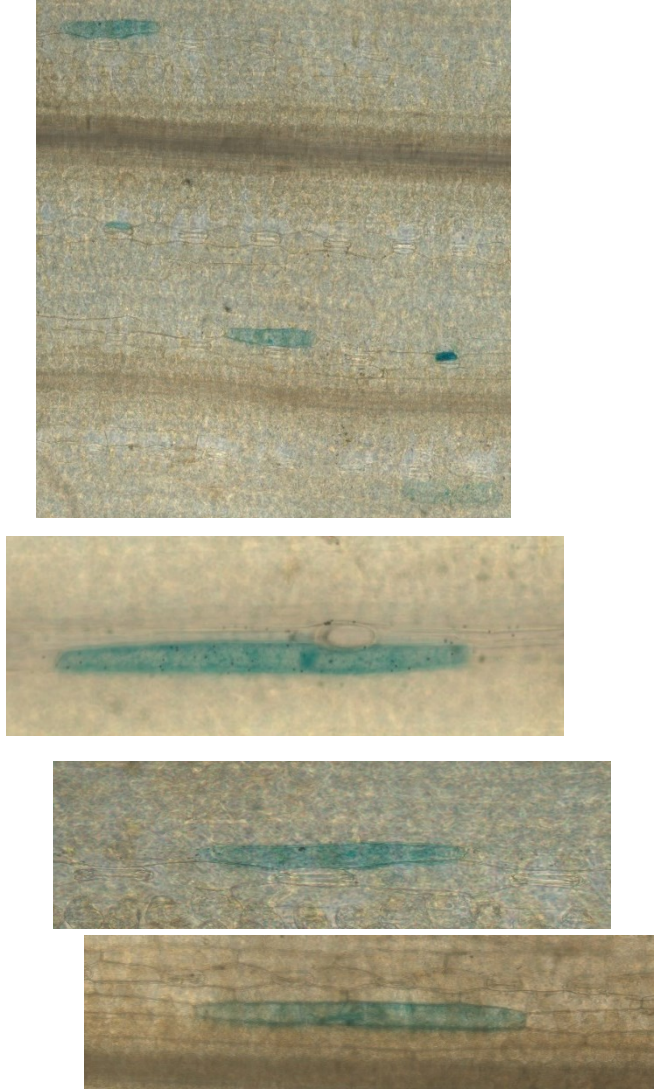
Overexpression of MLO gene in braley cells results in more susceptible cells infected by conidia of the powdery mildew fungus (*Blumeria graminis* f. sp. Hordei) compared to silencing of the gene in the cells in the folllowing slide

Our results showed that leaves of genotypes that were shot by overexpression constructs of MLO gene were very susceptible to the fungus because the gene in the plant cells was overexpressed and therefore fungal conidia could penetrate many cells and produce haustorium compared to the leaves only shot by reporter gene.

Also, genotypes shot with silencing construct were more resistant to the pathogen because the gene was silenced in the plant cells and therefore, the conidia of the fungus could not penetrate the cells



cells were shot with overexpression construct which overexpressed the MLG gene in the susceptible genotype of barley to the fungus. Almost all shot cells have been infected by conidia and the haustorium of the fungus penetrated the cells.



(B) silencing the gene (MLO) in the susceptible genotype of barley to powdery mildew fungus. Less cells or no cells have been infected with the fungus due to the silencing the susceptible gene to the fungus.

Conclusion

- The use of particle bombardment as a gene delivery system has proven to be a valuable tool in plant research,

- The comprehensive training provided by the host institute, including the lab experiments and protocols for gene delivery system and assessing gene function in cereals

- Continue exploring its potential to test gene function in **Fusarium-related research**

Acknowledgments

Special Thanks:

- Dr. Dimitar Douchkov and the IPK team for their guidance and support.
- STSM Grant for funding this invaluable training opportunity.
- My colleague at SLU for his support and collaboration.