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Development of an Ascochyta blight screening system *in vivo* and *in vitro* for the selection of resistant pea (*Pisum sativum* L.) accessions

Introduction

- Pea (*Pisum sativum* L.) has become important for sustainable cropping systems, nitrogen fixation and for human nutrition.
- Pea production is challenged by fungal pathogens, such as the species contributing to the Ascochyta blight complex (*Ascochyta pisi*, *Didymella pinodes,* and *D. pinodella*), particularly in organic or sustainable cropping systems with limited use of fungicides.
- The selection and breeding of resistant pea varieties is therefore one of the most promising approaches.
- This requires a reproducible and quantitative screening method for the phenotypic selection of resistant pea accessions and identification of resistance genes (GWAS).

Methods

- Fungi were grown on PDA.
- Pea seedlings (10d after sowing) were inoculated with conidia suspension at 10^5 conidia/ml (Fig. II A).
- Detached leaf assay: leaves were cut and placed on 2% water agar and then spray inoculated (Fig. II B).
- Disease assessment *in vivo*: the % of necrotic leaf surface was calculated as the mean of the three leaf pairs from the first to the third nodal level of the shoot axis (Fig. III A+B) and Fig IV).
- Disease assessment *in vitro*: in % of necrotic leaf surface.

Results

A simple and fast phenotypic screening of resistance was achieved with the scoring models. Clear differences between varieties were obtained in both *in vivo* (Fig. IV A+B) and *in vitro* (Fig. IV C+D).

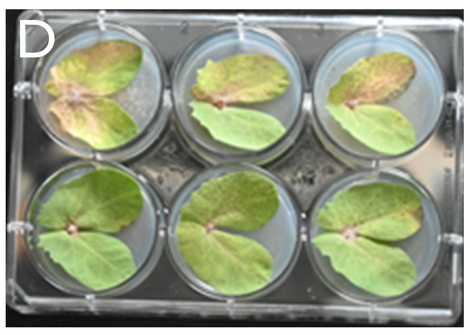

Fig. IV: Susceptible pea variety Hubal **A** *in vivo* and **C** *in vitro* method with leaf infestation greater than 50%. Less susceptible pea variety Turnia **B** *in vivo* and **D** *in vitro* with leaf infestation grade of around 20%

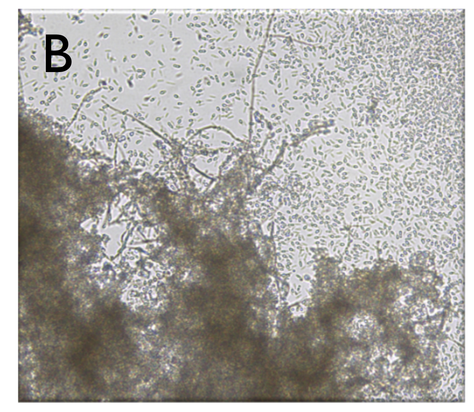

Fig. I: A Fungal isolation of infected plant, **B** Pure culture with *Ascochyta pisi* conidia.

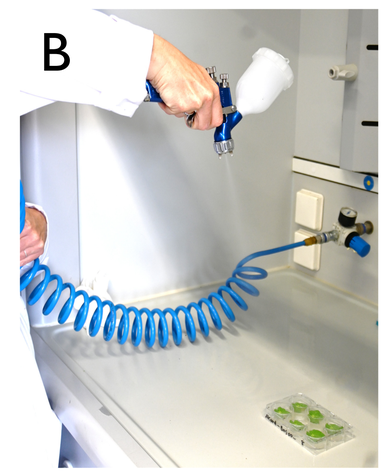

Fig. II: Spore inoculation with a high pressure spray gun (DeVilbiss) of (**A)** whole plants, (**B)** detached leaves on plates

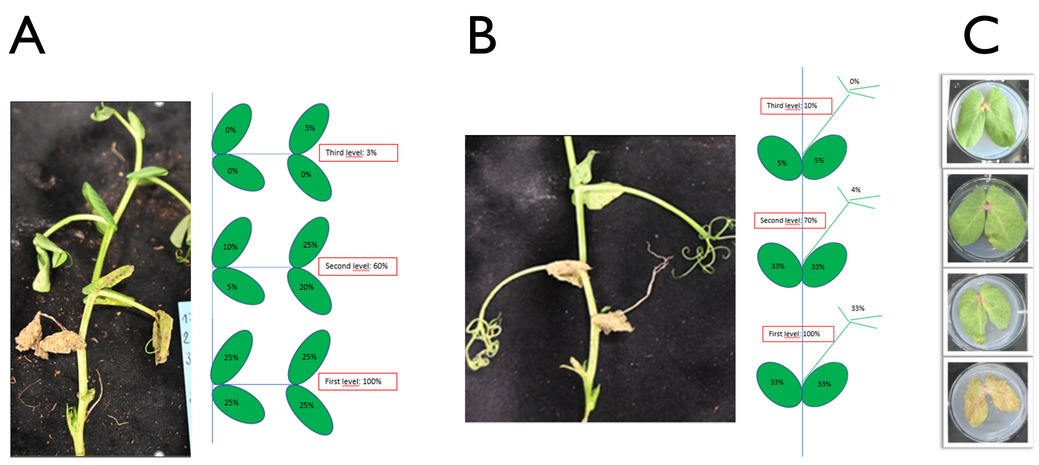

Fig. III: (A) 4-Leaves scoring model for leaf type varieties**, (B)** 2-Leaves scoring model for tendril type varieties, **(C)** Detached leaf assay from top to bottom**:** no infestation 0%, weak infestation >10%, medium infestation <40%, heavy infestation <80%.

Discussion and Conclusion

- The *in vivo* screening method is fully established and validated.
- Consistent datasets over several trials could be generated with clear different infestations levels between pea varieties.
- However, the results between *in vivo* and *in vitro* do not always correlate. One reason for this could be a wound-induced defense response of the detached leaves. In further steps, this will be investigated by comparing the gene expression level of pathogenesis related proteins (PR proteins).
- This method, in particular the detached leaf method has the potential for testing also larger series of accessions.

References
Annan, E.N.; Nyamesorto, B.; Yan, Q.; McPhee, K.; Huang, L. Optimized High Throughput Ascochyta Blight Screening Protocols and Immunity to *A. pisi* in Pea. Pathogens 2023, 12, 494. https://doi.org/10.3390/pathogens12030494

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