

Roots of Resistance: Insight into the *Verticillium dahliae* effector repertoire during xylem infection of potato

¹Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands

²Department of Plant and Microbial Biology, University of Zürich, Zürich, Switzerland

³Institute for Plant Sciences, University of Cologne, North Rhine-Westphalia, Cologne, Germany



Verticillium wilt in potato

Verticillium wilt is a disease affecting more than 400 different host species, ranging from annual crops to trees, and caused by the soilborne fungus *Verticillium dahliae* Kleb. Potato, the fourth most important staple food crop worldwide¹ is also affected by Verticillium wilt. The pathogen can severely reduce yields in potato fields² by blocking transport of water and clogging xylem vessels with conidia and fungal mycelium (Figure 1). On infected plant debris, *V. dahliae* produce resting structures called microsclerotia, making modern fungicides ineffective. An alternative source might be the use of natural resistance in wild potatoes. We aim to identify expressed effectors that might lead to the discovery of resistance (*R*) genes in wild *Solanum* species.

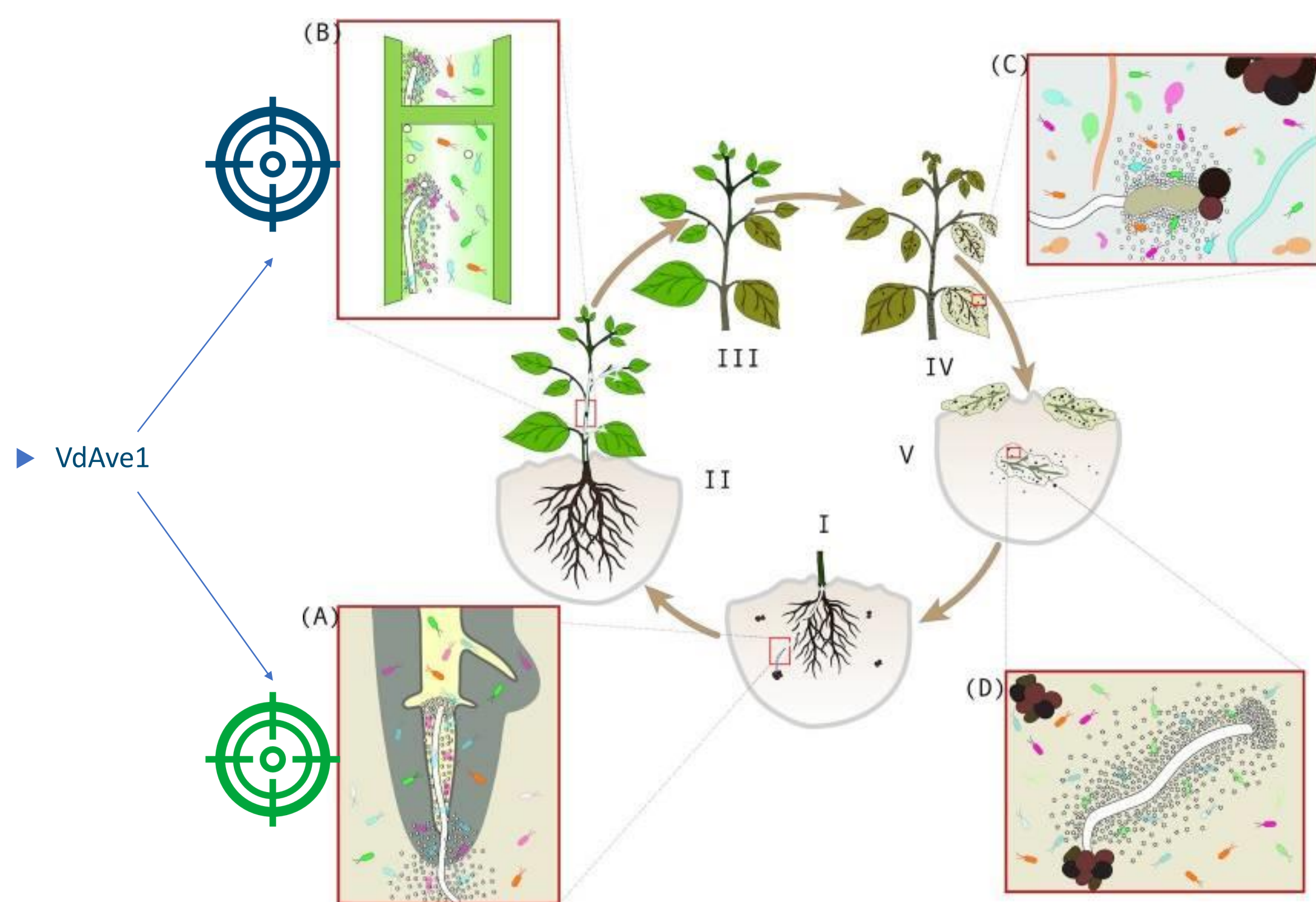


Figure 1: Disease cycle of *Verticillium dahliae* in potato and the role of effectors in pathogenicity. Once root exudates are perceived by microserotetria, *V. dahliae* enters via wounds in the roots. The pathogen penetrates until the xylem vessels of the host, where it will proliferate. Effectors play a role in the infection, and some effectors, such as VdAve-1, compete with soil microbiota (adapted from 3).

Xylem sap enrichment enables effector gene profiling during infection

Effector expression analysis is limited by the low biomass of *V. dahliae* in xylem vessels. To overcome this, we extracted xylem sap from susceptible potato and supplemented it into mycelial cultures, yielding sufficient RNA-Seq reads for analysis (Figure 2).

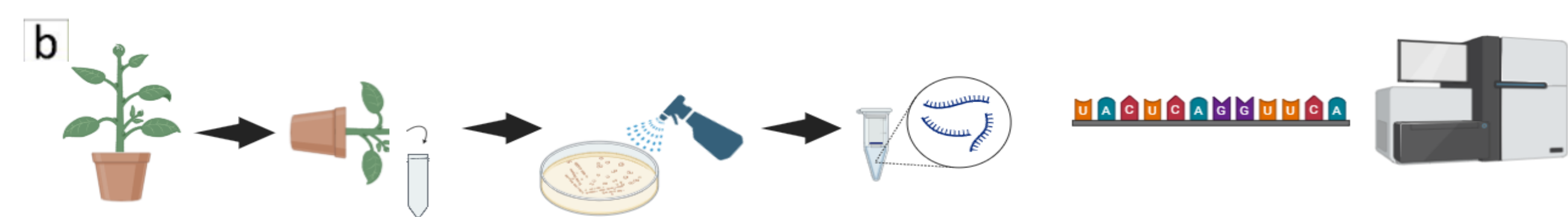
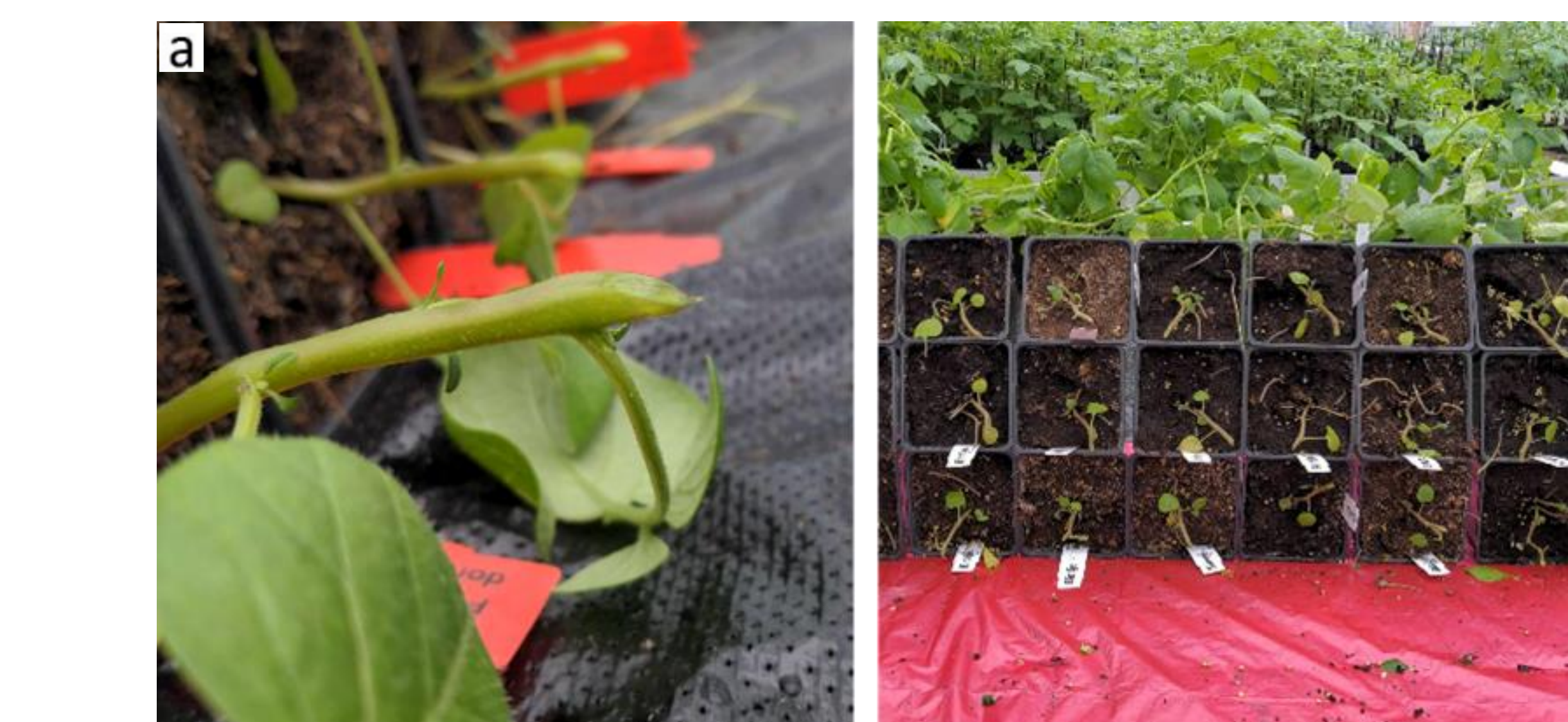


Figure 2: A) Plants were grown for one month in a greenhouse compartment, then were cut at 10 cm from the soil level and placed horizontally to facilitate the extraction of xylem sap. The first drop was washed away and subsequently xylem was collected in 50 mL tubes supplemented with Proteinase Inhibitor Tablets EDTA-free. B) Visual representation of the xylem extraction pipeline. After xylem extraction from a susceptible potato cultivar, *V. dahliae* is grown in Potato Dextrose Agar (PDA) for 14 days and sprayed either with xylem sap or synthetic xylem media. As control we used a PDA with mycelium without any spraying. The mycelium was collected; RNA was extracted and sent for sequencing.

Effector identification

Using our RNA-Seq dataset, we implemented an effector prediction pipeline and identified 98 secreted effectors from *V. dahliae* (Figure 3). Analysis of the biological features of these effectors revealed that 38 were predicted to have an antimicrobial activity, as determined by AMAPEC⁴.

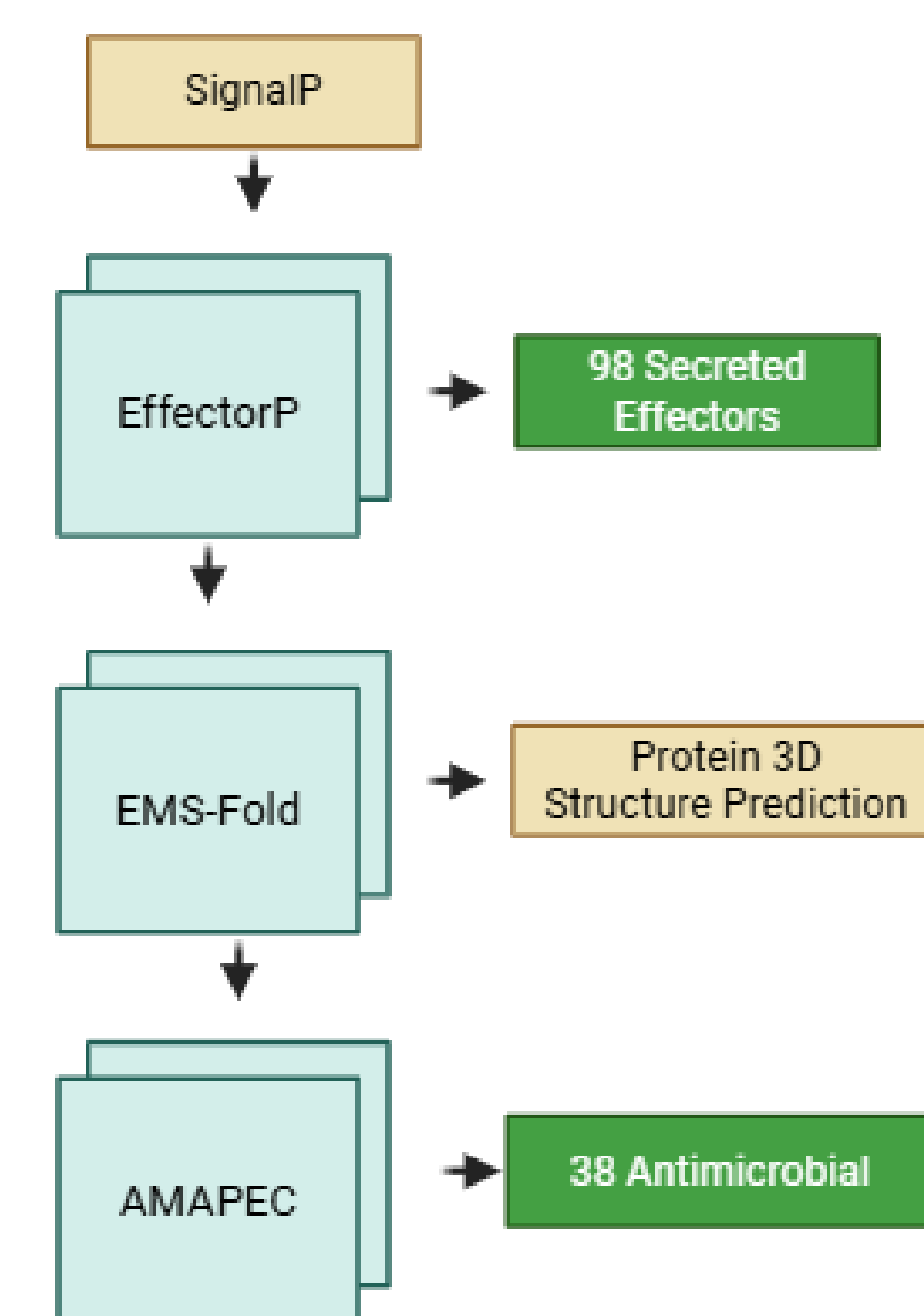


Figure 3: Effector prediction pipeline. EffectorP 3.0 was used to predicted effectors after differentially expressed genes (DEGs) analysis (DESeq 2, Rstudio) and AMAPEC for predicting antimicrobial activity.

Effector screening with PVX-agroinfection

To detect *R* genes against *V. dahliae*, we are subjecting resistant wild potatoes to effectoromics screens⁵. Genotypes that recognize VdAve-1 and VdAv-2 point to presence of *Ve1* and potentially a new resistance gene, respectively (Figure 4).

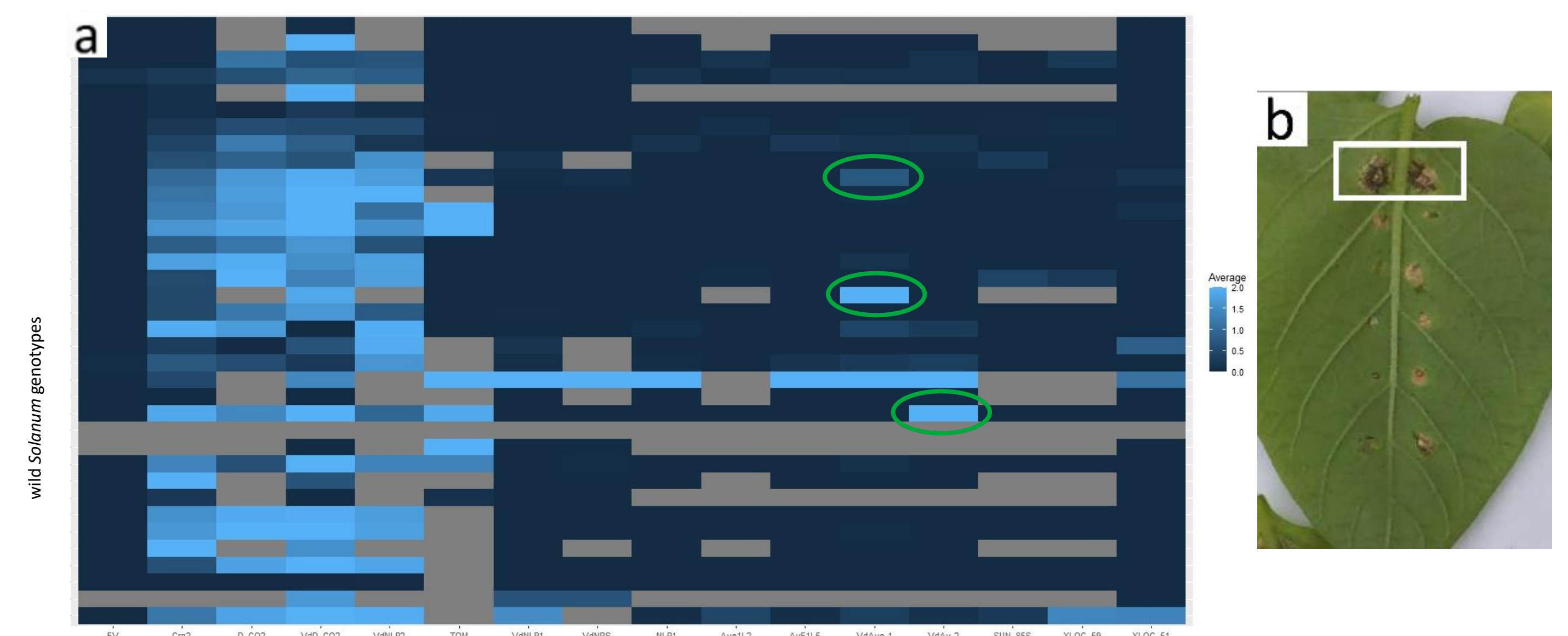


Figure 4: A) Wild *Solanum* genotypes were tested using PVX-agroinfection with effectors from *V. dahliae*. The heat map above shows specific level of cell death (0-2). NLP from *V. dahliae* and the non-specific cell death inducing protein Crn2 (+) from *P. infestans* were used as positive controls, while the empty PVX vector as negative control (-). B) Leaf of a resistant genotype with specific response to VdAve-1.

Conclusion

- We identified a set of expressed effectors of *V. dahliae* by simulating infection of potato plants with xylem sap.
- Approximately 40% of these effectors are predicted to have antimicrobial activity, suggesting a potential role in microbial competition.

Reference

- ¹ Food and Agriculture Organization (FAO). 2022. Role and Potential of Potato In Global Food Security
- ² Brown, R. M., Riedel and M.J. Martin. 1985. Synergic interactions between *Verticillium dahliae* and *Phytophthora blight* in potato early dying disease. *Phytopathology* 75 (In press)
- ³ Snelders NC, Rovenich H, Thomma BPHJ. Microbiota manipulation through the secretion of effector proteins is fundamental to the wealth of lifestyles in the fungal kingdom. *FEMS Microbiol Rev*. 2022 Sep 26;euj025;fuj022. doi: 10.1093/femsfure/fuac022. PMID: 35604874; PMCID: PMC9348471
- ⁴ Fantin Mesny, Bar Pui Thoma (2024). AMPARCC: accurate antimicrobial activity prediction for fungal effector proteins. *bioRxiv* 2024.01.04.574150; doi: <https://doi.org/10.1101/2024.01.04.574150>
- ⁵ Nischouline, C. G. C., MacFarlane S, Vossen, H. J., Champourou, N., Oliver, R., Segretni, M. M., (…), Kamoun, S. (2011). Understanding and Exploiting Late Blight Resistance in the Age of Effectors. *Annual Review of Phytopathology* 49(1), 507-531. doi: <https://doi.org/10.1146/annurev-phyto.08.06.10.0053>