Effector gene variation in Polish and Norwegian *Phytophthora infestans* strains

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Phytophthora infestans infects potato

The oomycete *Phytophthora infestans* causes **late blight**, a devastating potato disease (Fig. 1). The pathogen secretes effector proteins targeted to the host apoplast or to the interior of the plant cells to overcome the host's defence responses and establish infection. Effector repertoire and sequence variation drive virulence in *P. infestans* strains.

P. infestans can reproduce sexually or clonally. Currently, clonal lineages are commonly classified by genotyping based on length variations in Simple Sequence Repeats (SSRs) across 12 loci (Li et al. 2013). Genetic and phenotypic differences can occur between isolates from one clonal lineage (Abu-El Samen et al., 2003; Hansen et al., 2016).

We used amplicon sequencing to explore sequence variation in effector genes from clonal and sexual *P. infestans* strains from Poland and Norway.

Fig. 1: Symptoms of late blight caused by *P. infestans* on the underside of potato leaves

Amplicon sequencing (AmpSeq)

We developed two AmpSeq approaches optimized for the Illumina MiSeq and PacBio HiFi platforms. AmpSeq was used to investigate variation in effector genes, primarily from the RXLR family, as well as other genes in approximately 400 Norwegian and Polish isolates of *P. infestans*. In total, we targeted more than 50 effectors with largely overlapping sets for the two sequencing platforms and multiple primer pairs for some targets. For Illumina MiSeq, amplicons were limited to about 350 bp in length, while we aimed for amplification of full coding sequences for PacBio Sequel II. We implemented various bioinformatics tools to build a customized, fully open-source and reproducible R-based analysis pipeline. This largely automates the initial data processing and generates several tabular and figure outputs that guide deep exploration of the data.

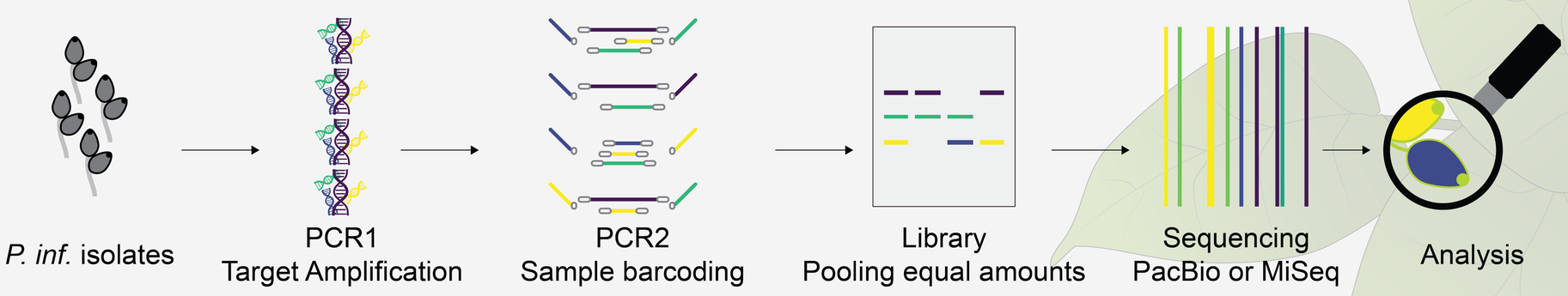

Fig. 2: Workflow illustration for amplicon sequencing of *Phytophthora infestans* isolates

AvrSmira1 and *PexRD2*-family variants in 13_A2 strains

Results: The reduced dataset shown here contained a total of 27 isolates that were categorized as genotype 13_A2 by SSR analysis. Variants of the two effector genes *AvrSmira1* and *PexRD2* were obtained from 25 and 24 isolates, respectively, and compared to publicly available sequences (Fig. 3). For **AvrSmira1**, AmpSeq of the partial coding sequence using the Illumina MiSeq platform was unsuccessful in a pilot run. Five *AvrSmira1* variants of the full coding sequence were obtained with AmpSeq using PacBio Sequel II for these isolates. For **PexRD2**, AmpSeq primers for Illumina and PacBio did not cover the whole coding sequence but both yielded amplicon sequence variants (ASVs). PacBio had superior coverage for *PexRD2* in these isolates as well.

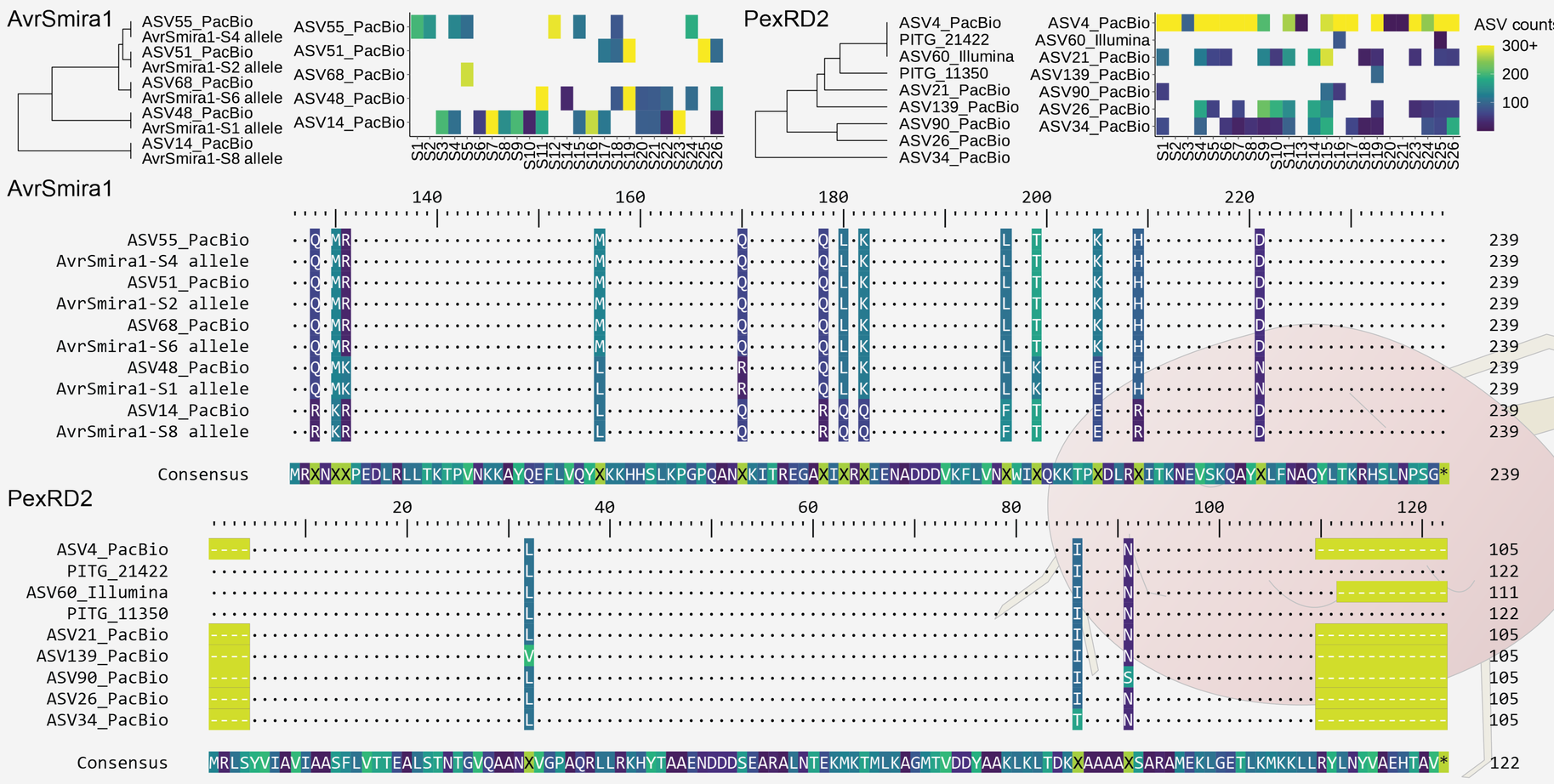

Fig. 3: Dendrograms of relationships between ASVs and reference sequences and tile map of ASV distribution in strains S1-S27 for *AvrSmira1* (top left) and *PexRD2* (top right). Protein sequence alignments for ASVs and reference sequences for AvrSmira1 (middle) and PexRD2 (bottom).

Discussion: For the isolates of 13_A2 and the two effectors shown here, AmpSeq revealed sequence variation and different combinations of the variants between isolates. All five ***AvrSmira1*** variants correspond to known alleles described in Stefańczyk et al. (2018) and code for three distinct proteins. Isolates S18 and S26 yielded three variants of *AvrSmira1*, suggesting possible triploidy. Of the six unique ASVs of ***PexRD2*** found here, ASV4_PacBio and ASV60_Illumina corresponded to *PexRD2* from the reference strain T30-4, which contains multiple homologues. As most isolates here contained three or four variants, it is likely that these represent multiple loci. The six detected variants code for three distinct proteins with one aa substitution each.

References:

Abu-El Samen et al. (2003). Phytopathology 93(3):293-304
Hansen et al. (2016). PLOS ONE 11(11): e0165690
Li et al. (2013). J Microbiol Methods 92(3):316-22
Stefańczyk et al. (2018). Plant Pathology 67:1792-1802

This cartoon illustrates sporangia of *Phytophthora infestans,* containing genomic differences, growing from lesions on symptomatic leaves of an infected potato plant. Illustrations not to scale.

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