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Pangenome of global *Phytophthora ramorum* lineages reveals loss of crinkler effector genes and intra-specific plasticity

Sudden oak death in North America and Sudden larch death in Europe are devastating diseases caused by Phytophthora ramorum. The recent discovery that P. ramorum is likely native to Asia has provided further opportunity to investigate its evolutionary history. We used newly assembled, highquality genome sequences of three Asian and three introduced US lineages of P. ramorum to carry out a pangenome analysis. We focused on comparing putative conserved effector genes. Crinkler genes are an ancient class of oomycete cytoplasmic effectors involved in host necrosis and expanded in most Phytophthora species. Apparently, crinkler genes underwent a recent contraction, with the loss of multiple crinkler families, in Phytophthora sub-clade 8c that contains P. ramorum. Furthermore, in the ancestor of both P. ramorum and its sibling species P. lateralis we detected a significant absence of paralogs in the largest core crinkler family. Secreted carbohydrate-active enzymes, believed to influence host range of fungal and oomycete plant pathogens, are just as variable in abundance within P. ramorum as among other clade 8c species. Although the relative abundance of certain effectors was variable among lineages P. ramorum lineages, there was little difference in presence or absence of entire families. This suggests that rather than effector novelty, it is possible that differences in dosage of certain effectors underlies variation in disease severity among lineages on diverse hosts. This work provides novel insights into the history of effector gene evolution.

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Extended methods

- •Sequenced one strain each of clonal lineages epidemic in US (NA1, NA2, EU1), three from candidate center of origin (Indochina/Vietnam: IC1, IC2; Nippon/Japan: NP1)
- •Assembled NA1, EU1, IC1, IC2, NP1 from PacBio data and NA2 from Nanopore
- •Scaffolded chromosomes with intra- and interspecific homology, curated suspicious joins
- •Gene annotation: BRAKER (Hoff et al. 2019), supplemented RXLR and crinkler effector candidates from all ORFs. RXLRs and crinklers annotated using custom pipelines with HMM and string searches of known motifs.
- •Glycoside hydrolase domains: dbCAN2 (Lombard et al. 2014)
- •Synteny: GENESPACE (Lovell et al. 2022)
- •Phylogenetics: Funannotate (Palmer and Stajich 2020) IQ-Tree on trimmed alignment of 118 single-copy BUSCO proteins (Nguyen et al. 2015, Seppey et al. 2019)
- •Orthogroups: OrthoFinder (Emms), visualized with UpSetR (Gehlenborg 2016)
- •Gene family birth-death: CAFÉ 5 (Mendes et al. 2020)
- •Gene annotation: BRAKER (Hoff et al. 2019), supplemented RXLR and crinkler effector candidates from all ORFs

Funding

This work was supported by grants from USDA ARS (2072-22000-041-000-D), USDA NIFA (2023-67013-39918) and USDA APHIS to N.J.G. and a USDA NIFA predoctoral fellowship 2021-67034-35171 to NC.

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