Prediction and Characterization of RXLR Effectors in *Pythium* Species

Gan Ai,¹ Kun Yang,¹ Wenwu Ye,¹ Yuee Tian,^{1,2} Yaxin Du,¹ Hai Zhu,¹ Tianli Li,¹ Qingyue Xia,¹ Danyu Shen,¹ Hao Peng,³ Maofeng Jing,¹ Ai Xia,¹ and Daolong Dou^{1,†}

¹ College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China

² Department of Plant Protection, Henan University of Science and Technology, Luoyang 471000, China

³ Department of Crop and Soil Sciences, Washington State University, Pullman, WA, U.S.A.

Accepted 23 April 2020.

RXLR effectors, a class of secreted proteins that are transferred into host cells to manipulate host immunity, have been reported to widely exist in oomycetes, including those from genera *Phytophthora*, *Hyaloperonospora*, *Albugo*, and *Saprolegnia*. However, in *Pythium* species, no RXLR effector has yet been characterized, and the origin and evolution of such virulent effectors are still unknown. Here, we developed a modified regular expression method for de novo identification of RXLRs and characterized 359 putative RXLR effectors in nine *Pythium* species. Phylogenetic analysis revealed that all oomycetous RXLRs formed a single superfamily, suggesting that they might have a common ancestor. RXLR effectors from *Pythium* and *Phytophthora* species exhibited similar sequence features, protein structures, and genome locations. In particular, there were significantly more RXLR proteins in the mosquito biological control agent *P. guiyangense* than in the other eight *Pythium* species, and *P. guiyangense* RXLRs might be the result of gene duplication and genome rearrangement events, as indicated by synteny analysis. Expression pattern analysis of RXLR-encoding genes in the plant pathogen *P. ultimum* detected transcripts of the majority of the predicted RXLR genes, with some RXLR effectors induced in infection stages and one RXLR showing necrosis-inducing activity. Furthermore, all predicted RXLR genes were cloned from two biocontrol agents, *P. oligandrum* and *P. periplocum*, and three of the RXLR genes were found to induce a defense response in *Nicotiana benthamiana*. Taken together, our findings represent the first evidence of RXLR effectors in *Pythium* species, providing valuable information on their evolutionary patterns and the mechanisms of their interactions with diverse hosts.

Keywords: gene evolution, oomycete, *Pythium*, pathogenicity, RXLR effector

Endless arms races between microbial pathogens and host plants are the main driving force of the coevolution of these organisms (Jones and Dangl 2006). Upon pathogen infection, plants trigger a series of defense responses by the recognition of conserved pathogen-associated molecular patterns (PAMPs) or avirulence effectors of pathogens with a diverse group of receptors (Monaghan and Zipfel 2012). As a counterdefensive tactic, numerous plant pathogens, such as bacteria, nematodes, fungi, and oomycetes, deliver a repertoire of effector proteins into hosts to suppress host immunity and facilitate infections (Jones and Dangl 2006; Latijnhouwers et al. 2003). RXLR proteins are a group of effectors initially identified from *Phytophthora* species that contain conserved RXLR-dEER motifs at N terminals, which function by entering host cells, and highly divergent C-terminals, which are associated with effector activity (Dou and Zhou 2012; Whisson et al. 2007). Many RXLR effectors have been reported to induce cell death in plants. For example, a broad survey of *Phytophthora sojae* RXLR effectors showed that 11 of the effectors could trigger cell death, chlorosis, or mottling in *N. benthamiana* leaves (Wang et al. 2011). Another effector, PcrXLR207, from *Phytophthora capsici*, induces host cell death to facilitate the transition from biotrophy to necrotrophy (Li et al. 2019). Additionally, some RXLRs are avirulence proteins that exhibit gene-for-gene interactions with specific host resistance proteins and trigger hypersensitive responses in plants (Anderson et al. 2015). Thus far, most studies of RXLRs have focused on members of genera *Phytophthora* and *Hyaloperonospora*.

To date, several bioinformatics approaches have been developed to identify RXLRs from *Hyaloperonospora* and *Phytophthora* species. A pilot method, which was designed based on the first 30 residues of signal peptides (SPs) and the following 30 residues of RXLR motifs, was used to characterize RXLRs in *Hyaloperonospora parasitica*, *Phytophthora ramorum*, and *Phytophthora sojae* (Win et al. 2007). Later, the hidden Markov model (HMM) in combination with regular expression (regex) methods searching for the RXLR-dEER string was used to identify RXLRs in three *Phytophthora* species (Whisson et al. 2007). A third RXLR-identification approach is the homology searching method, which was applied to predict 370 and 392 RXLRs in *Phytophthora ramorum* and *Phytophthora sojae*, respectively (Jiang et al. 2008). RXLR effectors were identified genome-wide in different *Hyaloperonospora* and *Phytophthora* species using the above three

Gan Ai and Kun Yang contributed equally to this work.

†Corresponding author: D. Dou, Email: ddou@njau.edu.cn

Funding: This project was supported by grants from the Fundamental Research Funds for the Central Universities (KYT202001 and JCQY201901), the National Natural Science Foundation of China (31625023 and 31721004) and the Special Fund for Agro-scientific Research in the Public Interest (201503112).

*The e-Xtra logo stands for “electronic extra” and indicates that a supplementary figures file and nine supplementary tables are published online.

The author(s) declare no conflict of interest.

© 2020 The American Phytopathological Society

methods with different thresholds. For example, the three approaches were combined to characterize RXLRs in *Plasmopara viticola*, a pathogen on grapevine (Yin et al. 2017).

RXLR effectors from other genera often contain a degenerate RXLR-dEER motif. Therefore, modified methods are most acceptable for the identification of RXLR effectors in species of genera other than *Hyaloperonospora* and *Phytophthora*. For instance, 26 Ac-RxL effectors were identified in *Albugo candida* by searching for the RXL string within proteins containing SPs. These Ac-RxLs induced cell death through effector activity, despite containing only a degenerate RXLR-dEER motif (Links et al. 2011). In *Albugo laibachii*, 25 RXLR and 24 RXLQ effectors were found, using similar methods, and two of the effectors were functionally analyzed (Kemen et al. 2011). An RXLR effector from a fish-infecting oomycete, *Saprolegnia parasitica*, was identified by searching SPs and RXLR strings and it functioned by entering the host cells to promote virulence (Wawra et al. 2012).

The genus *Pythium* belongs to order Pythiales, an order closely related to Peronosporales, Albuginales, and Saprolegniales (McCarthy and Fitzpatrick 2017). Unlike *Phytophthora* species, which are mostly plant pathogens, *Pythium* species have a wide range of hosts and occupy multiple ecological niches. Many *Pythium* species, including *P. ultimum*, *P. iwayamai*, and *P. aphanidermatum*, cause a wide variety of diseases in plants (Adhikari et al. 2013; Lévesque et al. 2010), whereas *P. insidiosum* is a notorious pathogen infecting humans and animals (Rujirawat et al. 2018). Interestingly, some *Pythium* species, such as *P. oligandrum* and *P. periplocum*, are used as mycoparasitic biocontrol agents, since they can infect fungal hosts and induce plant defense responses (Benhamou et al. 1997; Paul 1999; Takenaka et al. 2003). Likewise, *P. guiyangense* is a facultative mosquito pathogen and an effective biological control agent for management of mosquitos (Shen et al. 2019). Until now, no RXLR-encoding genes in *Pythium* spp. have been reported in published genome databases using HMM, regex, or modified methods (Adhikari et al. 2013; Lévesque et al. 2010; Rujirawat et al. 2018; Shen et al. 2019). For example, the HMM method with RXLR string searching was used to identify RXLR effectors in *Pythium ultimum*, but no RXLR candidates were characterized (Lévesque et al. 2010). The same conclusion was drawn in other *Pythium* genomes (Adhikari et al. 2013; Rujirawat et al. 2018). Therefore, it remains unclear whether *Pythium* genomes encode RXLR effectors. The molecular roles of RXLR effectors in the interactions between *Pythium* species and their broad hosts are underexplored.

In this study, we developed a modified method for de novo identification of RXLRs in the draft genome sequences of nine *Pythium* species and performed comprehensive genomic analysis on the predicted RXLRs. Our results suggested that *Pythium* RXLRs form a single superfamily with all other known oomycetous RXLRs and share similar sequence divergence, disorder content, and genome location features. Four novel RXLRs from *P. ultimum*, *P. oligandrum*, and *P. periplocum* were functionally analyzed, and our evidence demonstrated that they were functional RXLR effectors inducing plant cell death. Overall, our findings reveal the wide occurrence of RXLRs in *Pythium* species at the whole-genome level, demonstrate the effector activities of selected *Pythium* RXLRs, and provide a solid platform for investigating the diversified roles of *Pythium* RXLRs in pathogenicity.

RESULTS

De novo identification of RXLR effectors.

No *Pythium* RXLR effector has been previously identified, although numerous RXLRs have been reported in phylogenetically

close species (Adhikari et al. 2013; Lévesque et al. 2010; Rujirawat et al. 2018; Shen et al. 2019). Here, we developed a de novo identification method with a modified regex model to screen RXLR candidates in nine *Pythium* species (Fig. 1A). The well-studied genomes of *Phytophthora sojae*, *Phytophthora ramorum*, and *Hyaloperonospora arabidopsidis* were used as references, and two diatom genomes were also included in the analysis (Fig. 1B; Supplementary Table S1). All open reading frames (ORFs) were retrieved from each genome because almost all reported RXLR genes lack introns (Jiang et al. 2008). Sequences were considered as sORFs (ORFs encoding a putative secretory protein) if their encoding proteins contained an SP but lacked a transmembrane region (TM).

In total, 311,164 sequences were obtained, with 12,490 to 33,431 sORFs found in each *Pythium* species, similar to the numbers of sORFs in genera *Hyaloperonospora* and *Phytophthora* (12,830 to 25,178 sORFs). Interestingly, only 47 to 12,213 sORFs were identified in the nonoomycetes (Supplementary Table S1). Significantly more sORFs were found in oomycetes than in other species ($P < 2.2E-16$, Fisher's exact test), indicating the occurrence of more expansion events in secretory protein-encoding genes in oomycetes than in non-oomycetes.

Since some functionally verified RXLR effectors contain degenerate dEER motifs (Supplementary Table S2), the original regex model (SP.{1,96}R.LR. {1,40}[ED][ED][KR]) (Whisson et al. 2007) was modified (SP.{1,40}R.LR. {1,40}([ED].[ED][KR])[ED][ED]. {0,3}[KR]) (Fig. 1A) to allow matching of these atypical RXLRs. In total, 1,146 RXLR candidates were identified using this modified model (Fig. 1B). We found 63, 282 and 285 RXLRs in *H. arabidopsidis*, *Phytophthora ramorum*, and *Phytophthora sojae*, respectively. They are all subsets of previously identified RXLRs and accounted for 42, 53, and 42% of the total sets, respectively (Baxter et al. 2010; Jiang et al. 2008; Win et al. 2007). In contrast, only 0 to 9 RXLRs were predicted in fungi, nematodes, or bacteria. These control results demonstrate both the high reliability and the low false-positive rate of our method. Twenty-one to 78 putative RXLRs were identified in each *Pythium* species. All predicted *Pythium* RXLR proteins contained a conserved SP, an RXLR motif, and a dEER motif, in which two acidic amino acids (D or E) were enriched and were followed by a conserved basic amino acid (R or K) (Fig. 1C; Supplementary Fig. S1).

To test the reliability of RXLR prediction in *Pythium* spp., an enrichment assay was conducted by comparing the RXLR number in each oomycete species with the RXLR number in the fungal pathogen *Botrytis cinerea* (randomly selected as a control). All oomycetes, including the *Pythium* species, showed significant enrichment of RXLR sequences ($P < 0.01$, Fisher's exact test) (Supplementary Table S3). Enrichment was not observed in non-stramenopiles species ($P > 0.05$, Fisher's exact test). Next, a permutation test was performed to evaluate whether the predicted RXLR effectors occurred randomly. We permuted the 90 after-SP residues of all sORFs and re-performed the RXLR search with the modified model 100 times to estimate the false-positive rates. In general, few numbers RXLR predictions were generated from the permuted sequences, indicating that the vast majority of oomycete RXLRs were not predicted by chance (Supplementary Table S4). For example, 35 RXLRs were predicted in *P. oligandrum*, whereas only one RXLR was detected, on average, in its permuted sequences. In addition to their presence in all examined oomycetes, including *Pythium* species, RXLR sequences were also identified in diatoms (20 in *Thalassiosira pseudonana* and 16 in *Phaeodactylum tricornutum*) with significant enrichment ($P < 0.01$, Fisher's exact test). These sequences might be the

precursors of RXLR effectors that help diatoms compete with other organisms in complex environments.

The evolutionary patterns of oomycete RXLRs.

Despite the high divergence of RXLR proteins resulting from the host-pathogen arms race, RXLR effectors from *Phytophthora sojae* and *Phytophthora ramorum* exhibited significant relatedness, suggesting that hundreds of fast-evolving RXLRs in these two species might be derived from a single common ancestor (Jiang et al. 2008). Therefore, we used BLASTP

software to analyze whether the predicted *Pythium* RXLRs belonged to the same evolutionary theme. The highly similar SP regions were removed to avoid interference. Using a BLASTP E value <1 as the cutoff, 840 RXLR proteins had significant hits to one or more other RXLRs. In total, 15,071 RXLR pairs were identified that formed a large relatedness network (Fig. 2A). RXLRs from *Phytophthora* spp. had a higher within-genus relatedness level than those from genera *Pythium* and *Hyaloperonospora*, suggesting a larger contribution of recent gene expansion events in *Phytophthora* spp. than

Fig. 1. De novo identification of RXLR effectors in *Pythium* species. **A**, The RXLR effector identification pipeline. sORFs (open reading frames [ORFs] encoding a putative secretory protein) indicate ORFs encoding secretory proteins with a signal peptide (SP) but without a transmembrane region (TM). **B**, Summary of predicted RXLRs. The phylogeny of the 26 species is based on data from the Taxonomy Database and previous studies. The ratios of sORFs to whole ORFs, the counts of RXLR candidates, and the ratios of RXLRs to sORFs in each species are shown in boxplots following the species names. The genome size of each species is shown after the corresponding boxplot. **C**, Weblogo of the RXLR-dEER motifs of *Pythium* RXLR effectors.

in *Pythium* and *Hyaloperonospora* species. We found three clusters sharing little relatedness with *Phytophthora* RXLRs, which indicated relative evolutionary independence. Almost all RXLRs in these clusters were from *Pythium* spp. The three clusters were named cluster 1 (C1), cluster 2 (C2), and cluster 3 (C3) (Fig. 2A). For example, the average sequence identity of the 22 RXLRs in C1 was 54%. A Peptidase_S8 domain (PF00082.22), which was absent in *Phytophthora* spp., was found in the C-termini of 21 C1 RXLRs.

Conserved RXLRs may play critical effector roles in pathogen virulence (Deb et al. 2018). Analysis of shared sequence identity (peptides with BLASTP hit E value <1e-5 and identity >30%) revealed 67, 93, and 28 *Pythium* RXLRs conserved at an intraspecies, intragenus, and intergenus level, respectively (Supplementary Table S5), suggesting that *Pythium* RXLRs share a common ancestor with RXLRs from other genera and were only slightly expanded after species divergence. These conserved RXLRs may be essential effectors for pathogens and deserve more attention in future studies.

Phylogenetic analysis was performed to further elucidate the evolutionary relationships among oomycete RXLRs as well as RXLR-like proteins from diatoms. Due to the host-pathogen arms race, RXLR C-termini (after the dEER motif) are much more divergent (Win et al. 2007) than the relatively conserved N-termini of RXLRs (Jiang et al. 2008; Kale et al. 2010). Therefore, when building the phylogenetic tree, C-terminal and SP regions were discarded to reflect RXLR evolutionary relatedness with minimal interferences from host coevolution and secretory signal sequences. Except for PyoRXLR33 and HyarRXLR1, all other 1,111 RXLRs formed a large clade (bootstrap value = 100), indicating that they belong to a single superfamily. Using bootstrap value >80 as the criteria, 972 RXLRs were divided into 105 subfamilies with member sizes ranging from two to 112 (Supplementary Table S6). No significant divergence was detected among Peronosporales, Pythiales, Saprolegniales, and diatom RXLRs (Fig. 2B). Thirty-nine and six subfamilies contained 119 and 28 RXLRs exclusively from Peronosporales and Pythiales species, respectively. All other subfamilies harbored RXLRs from at least two different genera. Taken together, the phylogenetic results suggest a common ancestor shared by almost all RXLR and RXLR-like proteins, with species-specific expansion that was much less detected in *Pythium* spp. than in *Phytophthora* spp.

Rapid evolution and abundant disorder content of *Pythium* RXLRs.

The highly divergent RXLRs of *Phytophthora* and *Hyaloperonospora* species are among the most rapidly evolving portions of their proteomes (Jiang et al. 2008; Win et al. 2007). Similarly, *Pythium* RXLRs shared low identities in our study. On average, only 37% identity was detected between all closest RXLR ortholog pairs within the *Pythium* genus. In contrast, we randomly selected 300 *Pythium* proteins and found a much higher average sequence identity (67%) associated with their closest ortholog pairs (Fig. 3A). In our study, 233 (65%) *Pythium* RXLRs failed to form any ortholog pair, whereas only 10 of 300 randomly selected *Pythium* proteins lacked orthologs (Fig. 3A). Furthermore, 236 (66%) *Pythium* RXLRs showed more than 50% sequence divergence (Fig. 3A).

Phytophthora RXLRs have high disorder content, which contributes to their effector activities (Shen et al. 2017). Our analysis demonstrated that the disorder content in *Pythium* RXLRs was high as well. The mean percentages of disordered regions in randomly selected *Pythium* proteins, *Pythium* RXLRs, and *Phytophthora sojae* RXLRs were 27.17, 45.48, and 36.89%, respectively (Fig. 3B). *Pythium* RXLRs showed

obviously high disorder contents that were even higher than those of *Phytophthora sojae*. Over 64% *Pythium* RXLRs had 30% or more disordered residues, which can be found in only 43.67% of randomly selected *Pythium* proteins (Fig. 3B).

Pythium RXLRs are predominantly located in gene-sparse regions.

Phytophthora RXLR genes are preferably located in gene-sparse regions (Haas et al. 2009). Both the 5' and the 3' average intergenic distances of the *Phytophthora sojae* RXLR genes predicted in this study were significantly longer than those of the complete *Phytophthora sojae* genes ($P < 0.05$, Student's t test). The mean 5'-end intergenic distance of RXLR genes was 3.20 kb, with a whole-genome mean of 0.91 kb. The average RXLR 3' intergenic distance of 3.31 kb was also much longer than the 3' intergenic distance of complete *Phytophthora sojae*

Fig. 2. The evolutionary patterns of oomycete RXLR effectors. **A**, The relatedness network of putative RXLR effectors. Each spot represents an RXLR effector. Similar RXLRs (E value < 1) are joined by edges. Spot colors indicate genera. Three relatively independent clusters are marked as C1, C2, and C3. Almost all effectors in these clusters are from *Pythium* spp. The four RXLRs with effector activities verified in this study are labeled using arrows. **B**, Maximum-likelihood phylogenetic tree of the RXLR-dEER motifs in RXLR effectors. Gray, yellow, and red circles in the nodes indicate bootstrap values of 0 to 50, 51 to 80, and 80 to 100, respectively. Tree leaves are colored based on the families of their respective species.

genes (0.92 kb) (Fig. 3C). Both observations are similar to the results of a previous report (Haas et al. 2009).

Similar gene location preferences were detected for *Pythium* RXLRs in our study. At both the 5' and 3' ends, the average intergenic distances of the RXLR genes were longer than the average intergenic distances of the complete gene sets of all nine examined *Pythium* species (Fig. 3C). For the 5' and the 3' ends, this difference was statistically significant ($P < 0.05$, Student's *t* test) in seven and three *Pythium* species, respectively (Fig. 3C). These results indicate that *Pythium* RXLRs are predominantly located in gene-sparse regions, which is similar to the case of *Phytophthora* RXLR genes.

Genome rearrangement contributes to the emergence of novel RXLR genes in *P. guiyangense*.

The RXLR repertoire in *P. guiyangense* is larger than that in all other *Pythium* species examined (Fig. 1B). Our previous synteny analysis showed that *P. guiyangense* has a hybrid genome derived from two distinct parental species, which leads to a nearly twofold expansion of its predicted genes when

compared with other *Pythium* species. Consequently, most homologous gene pairs in *P. guiyangense* show high degrees of collinearity (Shen et al. 2019). However, we only found four RXLR genes from different scaffolds showing collinearity, indicating that additional mechanisms may have contributed to the expansion of RXLR genes in *P. guiyangense*. We noticed that 29 *Pygu*RXLR genes formed several clusters (<40 kb in size), with each cluster located in the rearrangement region of the same contig. A similar phenomenon was not observed in other *Pythium* species (Supplementary Fig. S2). For example, *Pygu*RXLR6/7/8 (Fig. 4A) and *Pygu*RXLR32/33/34 (Fig. 4B) were clustered in the genome rearrangement regions of contigs 2 and 9, respectively. Furthermore, 22 clustered RXLRs showed high similarities in the N-terminal region but had less conserved or even highly divergent C-termini within each cluster. For example, *Pygu*RXLR6, *Pygu*RXLR7, and *Pygu*RXLR8 shared a conserved N terminal but had highly divergent C termini (Fig. 4C). A similar pattern was observed in *Pygu*RXLR32 and *Pygu*RXLR33 versus *Pygu*RXLR34 (Fig. 4D). Taken together, these results suggest that both gene

Fig. 3. Evolutionary features of *Pythium* RXLR effectors. **A**, Sequence divergence of *Pythium* RXLR effectors. *Pythium* RXLRs were compared against 300 randomly selected *Pythium* proteins to determine the sequence identities of their closest ortholog pairs within the *Pythium* genus. Radiuses ranging from 0 (center) to 80 (outer circle) represent 100% to 20% (or less) sequence identities, respectively. Proteins with the same identity level are randomly distributed along their corresponding circle. **B**, Disorder content in *Peronosporales* and *Pythiales* RXLRs. The line plot shows the percentages of proteins containing different percentages of disordered regions. The bar plot shows the total disorder percentages in *Peronosporales* RXLRs, *Pythiales* RXLRs, and randomly selected proteins. RSP = randomly selected proteins, PyRXLR = *Pythiales* RXLRs, and PerRXLR = *Peronosporales* RXLRs. **C**, 5' and 3' intergenic distances of *Phytophthora sojae* and *Pythium* RXLRs. The two bar plots show 5' (left) and 3' (right) intergenic distances of the indicated RXLR genes. (one asterisk [*] indicates $P < 0.05$ and two [**] $P < 0.001$, Student's *t* test). Phso = *Phytophthora sojae*, Pyiw = *P. iwayamai*, Pyir = *P. irregulare*, Pyul = *P. ultimum*, Pyap = *P. aphanidermatum*, Pyar = *P. arrhenomanes*, Pyol = *P. oligandrum*, Pype = *P. periplocum*, Pygu = *P. guiyangense*, Pyin = *P. insidiosum*.

duplications and genome rearrangements contributed to the expansion and emergence of novel *RXL*R genes in *P. guiyangense*. Ultimately, the roles of *RXL*R effectors in *P. guiyangense*–mosquito interaction will be interesting for future study.

Most predicted *P. ultimum* *RXL*R genes are truly transcribed genes.

To further elucidate whether the predicted *Pythium* *RXL*Rs were truly transcribed genes, an RNA-seq analysis was carried out to examine the transcript accumulation of 40 *RXL*R candidates in *P. ultimum* during the mycelium stage and the stages at 3, 6, 12, 24, and 36 h after host infection. As one of the most important oomycetous plant pathogens with an available high-quality assembled genome sequence (Lévesque et al. 2010; Kamoun et al. 2015), *P. ultimum* was selected as the investigation target. Except for two candidates (PyuRXLR25 and PyuRXLR26) whose RPKM (reads per kilobase transcript length per million reads mapped) values were consistently <1 at all stages, transcripts of the remaining 38 predicted *RXL*R genes were detected at one or more stages (Fig. 5A), suggesting that most of the *P. ultimum* *RXL*R genes predicted in our study were truly transcribed genes.

P. ultimum *RXL*R genes showed stage-specific expression patterns. They were assigned to four gene expression clusters (Fig. 5A) via hierarchical clustering. C1 contained two putative pseudogenes and five *RXL*R genes exhibiting zero to low expression. Despite their low transcript accumulation, all *RXL*R genes in this cluster showed elevated expression during early infection stages. The 14 *RXL*R genes in C2 exhibited elevated transcript accumulation in both the mycelium and late-infection stages. The expression of 11 C3 *RXL*R genes was largely constant across all stages, with slightly more transcripts detected 3 to 6 h postinfection. The eight cluster 4 (C4) *RXL*R genes showed a similar expression pattern as the *RXL*R genes in

C2 but with much higher RPKM values. These results revealed major transcription shifts of *PyuRXLR* genes at the mycelium and different host-infection stages.

PyuRXLR22 induces cell death in *Nicotiana benthamiana*.

Phytophthora *RXL*Rs suppress PAMP-induced plant cell death. Furthermore, their ectopic expression can trigger cell death in plants (Wang et al. 2011). To evaluate the effector activity of predicted PyuRXLRs, we selected four candidates (PyuRXLR3, PyuRXLR4, PyuRXLR10, and PyuRXLR22, two candidates from C1 and two candidates from C4) for functional characterization (Fig. 5A). When coexpressed with *INF1* (Kanneganti et al. 2006) in *N. benthamiana* leaves, none of these four *RXL*Rs suppressed cell death triggered by the *P. infestans* PAMP INF1 (Supplementary Fig S3). In the evaluation of their ability to trigger host cell death by ectopic expression, only PyuRXLR22 induced dramatic cell death in *N. benthamiana* leaves (Fig. 5B). *PyuRXLR22* expression was highly upregulated at the late infection stages (Fig. 5A). Fused with a green fluorescent protein (GFP) tag at the N-terminal region, the four PyuRXLRs were transiently expressed in *N. benthamiana* epidermal cells to reveal their subcellular localizations. PyuRXLR22 was located in the endoplasmic reticulum (ER), plasma membrane, and nucleus (Fig. 5C). In contrast, PyuRXLR3, PyuRXLR4, and PyuRXLR10 were found in the plasma membrane and the nucleus but not in the ER (Fig. 5C). Bioinformatic analysis showed that PyuRXLR22 had two homologs in *Phytophthora ramorum* (BLASTP E value < 1) (Fig. 2A). The demonstrated effector activity makes PyuRXLR22 and its homologs promising targets for further investigation.

RXLRs from biocontrol *Pythium* species induce plant defense in *N. benthamiana*.

P. oligandrum and *P. periplocum* are two unique *Pythium* species that are widely used for controlling soilborne plant

Fig. 4. Synteny analysis and protein sequence alignments of clustered RXLRs in *Pythium guiyangense*. **A** and **B**, Synteny analysis of clustered *P. guiyangense* RXLRs. Red blocks indicate the clustered *PyguRXLR* genes described in the text. Green blocks indicate genes flanking these *PyguRXLR*s. Homologous genes with similar and opposite genome orientations are joined with black and red edges, respectively. **C** and **D**, Protein sequence alignments of two RXLR clusters. Identical or similar residues are colored in blue. The RXLR-dEER motifs are labeled in the alignments.

diseases. These species can infect pathogens and induce plant defense reactions (Benhamou et al. 1997; Paul 1999; Takenaka et al. 2003). To elucidate whether the RXLRs of *P. oligandrum* and *P. periplocum* participate in this biocontrol process, all 73 RXLRs of the two species were cloned and were expressed in *N. benthamiana* leaves individually to test their ability to trigger cell death. PyulRXLR32, PyeRXLR22, and PyeRXLR24 triggered cell death 7 days postinfiltration (Fig. 6A), indicating that the effectors may confer plant defense-inducing activity. All other 70 RXLRs lacked this ability. PyulRXLR32 was located in the plasma membrane and the nucleus. Both PyeRXLR22 and PyeRXLR24 were located in plasma and nuclear membranes (Supplementary Fig. S4).

To investigate whether the three RXLR effectors can induce plant defenses, we infiltrated *N. benthamiana* leaves with *Agrobacterium tumefaciens* harboring GFP or the indicated RXLR fusion construct and then inoculated the leaves with *Phytophthora capsici*. Compared with the GFP control, both PyulRXLR32 and PyeRXLR22 significantly reduced the lesion area caused by *Phytophthora capsici*, whereas PyeRXLR24 did not have a noticeable effect on lesion control (Fig. 6B and C).

Reactive oxygen species (ROS) play a central role in plant immunity and ROS bursts are a defense-response indicator (Qi et al. 2017). Our diaminobenzidine (DAB)-staining assay showed that, compared with the GFP control, ectopic

Fig. 5. Transcription patterns of *Pythium ultimum* RXLR genes and the effector activity of PyulRXLR22. **A**, Transcription patterns of *P. ultimum* RXLR candidates. The heat map shows gene transcription patterns at the mycelial (MY) stage and 3, 6, 12, 24, and 36 h postinoculation (hpi) of soybean leaves. Colored bars indicate the $\log_2$ of gene RPKM values (reads per kilobase transcript length per million reads mapped), ranging from dark blue (0) to red (13). Black bars indicate there was no expression detected. RXLRs selected for the effector activity assay are marked with arrows, and red arrows indicate the ability to induce cell death in *Nicotiana benthamiana*. Based on transcript accumulation levels and patterns, the *P. ultimum* RXLR genes are divided into four clusters, using hierarchical clustering methods and MeV software. Clusters are marked with circled numbers (1 to 4). **B**, PyulRXLR22 induced cell death in *N. benthamiana*. *N. benthamiana* leaves were transformed with the indicated constructs by agroinfiltration. Dashed circles indicate infiltration sites. The ratios indicate the number of sites with necrotic lesions versus the total number of infiltration sites. PyulRXLR3, PyulRXLR4, and PyulRXLR10 did not induce cell death. Green fluorescent protein and INF1 were used as negative and positive controls, respectively. **C**, Subcellular localization of PyulRXLRs. Green fluorescence indicates the localizations of PyulRXLRs in *N. benthamiana* epidermal cells. Photographs were taken at 48 hpi. Bar = 20 μ m.

expression of any of the three RXLRs induced an ROS burst in *N. benthamiana* leaves (Fig. 6D). Furthermore, all three RXLRs enhanced the ROS burst triggered by *Phytophthora capsici* inoculation (Fig. 6E).

Interestingly, PyoRXLR32 is conserved both intragenously and intergenously (Supplementary Fig. S5). Its *P. infestans* homolog (BLASTP E value = $2e-115$), previously reported as SF14 (suppressor of early Flg22-induced immune response 4), can inhibit host immunity and promote infection (Zheng et al. 2014); this role is opposite to the positive role of PyoRXLR32 in triggering cell death and defense responses. Likewise, although PyeRXLR21, PyeRXLR22, and PyeRXLR24 share high sequence similarity (Supplementary Fig. S6), PyeRXLR21 cannot trigger host cell death as PyeRXLR22, and PyeRXLR24 did (Fig. 6A). These results indicate that slight sequence differences may lead to diverse or even contrary functions of homologous RXLR effectors.

DISCUSSION

In this study, we performed a de novo identification of RXLR effector genes from the genomes of nine *Pythium* species and analyzed their evolutionary features. Selected *Pythium* RXLR effectors were functionally verified. Despite the modified prediction method we adopted, RXLRs were found in a wide range of oomycetes, including the studied *Pythium* species, but were almost absent outside stramenopiles species. Our phylogenetic

analysis indicates RXLRs have a common ancestor, with all oomycetous RXLRs clustered into a single superfamily. *Pythium* and *Phytophthora* RXLR genes share several features, including a rapid evolution, high disorder content and the tendency to be located in gene-sparse regions. In particular, the RXLR reservoir of *P. guiyangense*, due to frequent gene duplication and genome rearrangement events, is much larger than that of other *Pythium* species. Using *P. ultimum* as a model for plant pathogens, most of its predicted RXLR genes were demonstrated to be truly transcribed genes clustered into four distinct expression patterns. The effector activity of PyuRXLR22 was functionally verified in this study. Finally, we identified three functional RXLR effectors from two bio-control *Pythium* species.

Although no *Pythium* RXLRs have been identified before (Lévesque et al. 2010; Adhikari et al. 2013), there have been clues indicating their existence. Several RXLR effectors have been experimentally validated in species phylogenetically close to *Pythium*, including *Albugo candida*, *Albugo laibachii*, and *Saprolegnia parasitica* (Kemen et al. 2011; Links et al. 2011; Wawra et al. 2012). The necrotrophic infection type of *Pythium* pathogens also matches the observations that many RXLR effectors induce necrosis. Largely due to the stringent definition of the dEER motif, existing genome annotation models may be inadequate for RXLR gene prediction in *Pythium* species. Hence, we developed a modified regex model to allow the search for degenerate dEER motifs. Previously, reported

Fig. 6. RXLRs from *Pythium oligandrum* and *P. periplocum* induce plant defense responses. **A**, Expression of PyoRXLR32, PyeRXLR22, or PyeRXLR24 induced cell death in *Nicotiana benthamiana* leaves. *N. benthamiana* leaves were transformed with the indicated constructs by agroinfiltration. Dashed circles indicate infiltration sites, and the ratios indicate the number of sites with necrotic lesions versus the total number of infiltration sites. INF1 and the RXLR effector RXLR207 from *Phytophthora capsici* were used as positive controls. An empty vector expressing green fluorescent protein (GFP) was used as a negative control. The same controls were also used in the H₂O₂ accumulation assay. **B** and **C**, *Phytophthora capsici* infection was reduced by PyoRXLR32 and PyeRXLR22 but not by PyeRXLR24. At 24 h after infiltration, equal amounts of mycelium were inoculated onto each infiltrated area expressing PyoRXLR32, PyeRXLR22, or PyeRXLR24. Photographs were taken under UV light at 48 h postinoculation (hpi), and the lesion areas were measured at the same time. Lesion area data shown in the dot plots were collected from three biological replicates. At least five leaves were inoculated for each replicate (two asterisks [**] indicate $P < 0.001$, Student's *t* test). Bar = 1 cm. **D**, H₂O₂ accumulation in *N. benthamiana* leaves expressing PyoRXLR32, PyeRXLR22, or PyeRXLR24. Diaminobenzidine (DAB) staining was performed 48 hpi. Red dashed circles indicate infiltration sites. **E**, H₂O₂ accumulation in *Phytophthora capsici*-inoculated *N. benthamiana* leaves expressing PyoRXLR32, PyeRXLR22, or PyeRXLR24. *Phytophthora capsici* was inoculated 24 h after infiltration. DAB staining was performed at 12 hpi. Black dashed circles indicate inoculation sites.

effectors were adopted as references, and all six ORFs were retrieved from the whole genomes. To reduce false-positive rates, we omitted the BLASTP step following the RXLR search. By adopting our modified regex model, we predicted 359 new RXLR proteins in nine *Pythium* species and re-identified RXLRs from *H. arabidopsidis*, *Phytophthora ramorum*, and *Phytophthora sojae*. The high accuracy of our new model was demonstrated by the observation that very few RXLRs were predicted outside the stramenopiles and that all re-identified RXLRs were subsets of previously known RXLRs. Notably, our detection of RXLR-like proteins in diatoms suggests oomycetous RXLRs originated from an early ancestor of the stramenopile lineage. *Pythium* and other oomycetous RXLRs formed a superfamily in our phylogenetic analysis, which also supports the common ancestor hypothesis of RXLR evolution.

Despite the high divergence of RXLR sequences, the oomycetous RXLRs identified in our study were similar enough to form a large relatedness network, with *Phytophthora* RXLRs exhibiting the most overall sequence similarities. There were also RXLR clusters predominantly associated with *Pythium* or *Hyaloperonospora* species, suggesting these effectors evolved independently.

Phytophthora and *Pythium* RXLRs share several common features. *Phytophthora* RXLR effectors promote virulence by manipulating host defense processes. Thus, they undergo rapid sequence evolution to escape the surveillance of the plant immune system. In our study, this fast evolution pattern was also observed in *Pythium* RXLRs, which indicates their participation in host pathogenesis. As a consequence of effector evolution, there are abundant disorder regions in *Phytophthora* RXLRs (Shen et al. 2017). These disordered regions are even more prevalent in *Pythium* RXLRs. *Phytophthora* genomes feature the so-called ‘two-speed’ structure of gene-dense and gene-sparse regions (Raffaele and Kamoun 2012), and RXLR genes are enriched in the more dynamic gene-sparse regions. Gene-sparse regions are the hot sites of genome rearrangement and tend to accelerate pathogen evolution (Dong et al. 2015). In *Pythium* species, we also found that both the 5' and 3' average intergenic distances of RXLR genes were significantly longer than the whole-genome averages, which demonstrates *Pythium* RXLRs also tend to be located in gene-sparse regions.

Due to their highly divergent C termini, few RXLR paralogs were found in the *Pythium* genus, except for *P. guiyangense*. We characterized the *P. guiyangense* genome as a hybrid and identified a large set of RXLR genes. *P. guiyangense* RXLRs share low degrees of synteny with *PyguRXLR* paralogs, which are often clustered in the same contig and located in genome rearrangement regions. Furthermore, physically close paralogous pairs generally share a conserved N terminal but have less-conserved or even highly divergent C-termini. Our results indicate that recent gene duplication and genome rearrangement events shaped the evolution of *P. guiyangense* RXLRs. Previous research suggests that the duplications of genes encoding virulence-associated effectors facilitate pathogen adjustment toward host jumps (Raffaele et al. 2010). In the case of *P. guiyangense*, the detected duplication and rearrangement events may have generated novel RXLR genes helping the adaptation of the species against insects. RXLR genes also cluster closely (<100 kb) in *Phytophthora ramorum* (Win et al. 2007), suggesting that the RXLR gene cluster may be a common host adaptation strategy shared by oomycete species.

Our RNA-seq analysis of *P. ultimum* demonstrated that most predicted RXLR genes were transcribed genes, which further verified the reliability of our redesigned RXLR annotation method. Furthermore, these RXLR genes exhibit distinct expression patterns during infection. The expression stages of RXLRs are associated with their specific functions. In *Phytophthora*

sojae, RXLRs that are induced early inhibit host cell death triggered by PAMPs, while necrosis is caused by late-induced RXLR effectors (Wang et al. 2011). Another *Phytophthora* pathogen, *Phytophthora capsici*, secretes an RXLR effector to induce host defense responses and cell death and trigger the biotrophy-to-necrotroph transition (Li et al. 2019). Since most *Pythium* pathogens are necrotrophic, their RXLRs may trigger host cell death instead of acting as cell-death repressors. Consistent with this expectation, none of our tested *PyuRXLRs* suppressed INF1-induced host cell death. Instead, ectopic expression of *PyuRXLR22* triggered necrosis. The functional diversity of RXLRs in *Phytophthora* and *Pythium* species may result from their different nutrient acquisition strategies during infection.

P. oligandrum and *P. periplocum* are two biocontrol agents with largely unknown defense-inducing mechanisms. Previous reports suggest that plant defense may be induced by microbe-associated molecular patterns in these two *Pythium* species (Benhamou et al. 2012). In this study, we identified three RXLR effectors from *P. oligandrum* and *P. periplocum* and demonstrated the plant defense-induction ability of these RXLRs. This finding unveils the participation of cytoplasmic effectors in triggering host defense reactions. Since the *P. oligandrum* hyphae can penetrate into root tissues, the RXLR effectors of this species may be secreted to host cells via a similar mechanism as that detected in *Phytophthora* pathogens.

In conclusion, we identified RXLR effectors in *Pythium* species for the first time at the genome-wide scale. Their evolutionary features, sequence characteristics, and expression patterns were analyzed in detail. The effector activities of selected *Pythium* RXLRs, including their diversified functions in inducing cell death and host defense, were experimentally verified. This study expands our current understanding of oomycetous RXLR effectors to the *Pythium* genus. The resources generated in this work will facilitate future in-depth investigations on the interactions between the novel *Pythium* RXLRs and their diversified hosts.

MATERIALS AND METHODS

Data sets.

The genome sequences of *Phytophthora ramorum* and *Phytophthora sojae* (Tyler et al. 2006) were obtained from the Joint Genome Institute website. The genome sequences of *H. arabidopsidis* (Baxter et al. 2010), *Phytophthora vexans* (Adhikari et al. 2013), *P. aphanidermatum* (Adhikari et al. 2013), *P. arrhenomanes* (Adhikari et al. 2013), *P. irregulare* (Adhikari et al. 2013), *P. iwayamai* (Adhikari et al. 2013), *P. oligandrum* (Kushwaha et al. 2017), *P. periplocum* (Kushwaha et al. 2017), *P. ultimum* (Lévesque et al. 2010), *P. insidiosum* (Rujirawat et al. 2018), *P. guiyangense* (Shen et al. 2019), *Saprolegnia parasitica* (Jiang et al. 2013), *Phaeodactylum tricornutum* (Bowler et al. 2008), *Thalassiosira pseudonana* (Armbrust et al. 2004), *Saccharomyces cerevisiae* (Jacq et al. 1997), *Sclerotinia sclerotiorum* (Amselem et al. 2011), *Ustilago maydis* (Kämper et al. 2006), *Puccinia graminis* (Duplessis et al. 2011), *Botrytis cinerea* (Van Kan et al. 2017), *Caenorhabditis elegans* (*C. elegans* Sequencing Consortium 1998), *Meloidogyne hapla* (Opperman et al. 2008), *Escherichia coli* (Blattner et al. 1997), *Erwinia amylovora* (Smits et al. 2010) and ‘*Candidatus* Liberibacter asiaticus’ (Duan et al. 2009) were obtained from the National Center for Biotechnology Information. Detailed genomic information for all species is listed in Supplementary Table S7.

Identification of RXLR effectors.

For RXLR effector identification, all ORFs encoding proteins longer than 50 amino acids were retrieved. SPs were

predicted using SignalP v3 with the criteria that cleavage sites had to be located between residues 10 and 40 and HMM probability ≥ 0.9 (Bendtsen et al. 2004). TMs were predicted using TMHMM v2.0 (Krogh et al. 2001). Sequences containing SPs but lacking a TM were retained and were screened for RXLRs with a modified regex model described as SP.{1,40}R.LR.{1,40}([ED].[ED][KR][ED][ED].[0,3]KR). All matched protein sequences were predicted to be RXLR effectors. For redundant RXLRs caused by a set of alternative overlapping ORFs within the same reading frame, only the ORF encoding the longest RXLR was selected.

Permutation test.

A total of 90 after-SP residues of all sORFs were retrieved, permuted with random.shuffle() module in Python. Then the RXLR search using the method mentioned above was conducted in these permuted sequences. This process was conducted 100 times and the average number of RXLR effectors predicted in the permuted sequences was considered as the false-positive rate. The permutation code can be downloaded from GitHub.

Phylogenetic analysis.

For phylogenetic analysis, the SPs and C termini after the dEER motif were removed from the RXLR sequences. The truncated sequences were aligned using MUSCLE (Edgar 2004) and were then manually edited. Maximum-likelihood trees were constructed using IQ-TREE software with the -bb 1000 parameter (Nguyen et al. 2015). VT+F+R6 was selected as the best-fit model for this assay by using the ModelFinder tool in IQ-TREE. The phylogenetic tree was visualized using EvolView v3 (Subramanian et al. 2019).

Disorder region prediction and synteny analysis.

Disorder region prediction was performed by using ESpritz with the X-ray prediction type and the best-sw threshold (Walsh et al. 2012). Synteny analysis was conducted using MCScanX (Wang et al. 2012).

Vector construction.

For transient expression in *N. benthamiana*, *PyulRXLR*, *PyoRXLR*, and *PypeRXLR* genes (lacking the SP-encoding region) were amplified from their corresponding genomic DNA and were inserted into the pBinGFP2 vector (Song et al. 2015). The primers used for PCR amplification are listed in Supplementary Table S8.

Plant materials and agroinfiltration.

N. benthamiana plants were maintained in a greenhouse with an environmental temperature of 25°C under a 16-h light and 8-h dark photoperiod. The *A. tumefaciens* strain GV3101 had previously been stored in our lab. Before infiltration, *A. tumefaciens* clones harboring the indicated constructs were cultured at 28°C and 200 rpm for 48 h to reach the appropriate concentration. The *Agrobacterium* cultures were then resuspended in 10 mM MgCl₂ to an appropriate optical density (OD). For the necrosis induction assay, *A. tumefaciens* cells were infiltrated into the 6-week-old leaves of *N. benthamiana* at a final OD₆₀₀ of 0.2 for each construct. For the cell death suppression assay, *A. tumefaciens* cells expressing the indicated RXLR effectors (OD₆₀₀ = 0.3) were infiltrated into *N. benthamiana* leaves 1 day before the infiltration of *A. tumefaciens* expressing INF1 (OD₆₀₀ = 0.2). Symptom development was examined 7 days after infiltration.

Oomycete culture conditions and the inoculation assay.

The *P. oligandrum* CBS 530.74 and *P. periplocum* CBS 532.74 strains were provided by L. J. Grenville-Briggs at the

Swedish University of Agricultural Sciences and were stored at 25°C on 10% (vol/vol) V8 juice medium in the dark. *P. ultimum* and the *Phytophthora capsici* LT263 strain had previously been stored in our lab and were grown at 25°C on V8 medium in the dark. For *Phytophthora capsici* mycelium inoculation, 7-mm disks of 4-day growth medium were inoculated on *N. benthamiana* leaves 24 h after infiltration. Inoculated leaves were photographed at 48 h postinoculation (hpi) under UV light. Lesion areas were measured at the indicated timepoints. This assay was independently repeated at least three times. For the inoculation of *P. ultimum*, 4-mm plugs from the growing edge of a 2-day V8 agar culture were placed in the *N. benthamiana* leaves and the samples were harvested at the indicated timepoints.

Transcriptome sequencing and bioinformatic analysis.

For mycelium samples, *P. ultimum* mycelium growing in V8 agar culture for 2 days were harvested. *P. ultimum* samples at different infection stages (3, 6, 12, 24, and 36 hpi) were harvested using a 5-cm punch. Three biological replicates were performed and were used to produce an independent pool. The quality of total RNA was evaluated by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, U.S.A.). Samples with OD_{260/230} ≥ 1.8 and OD_{260/280} ≥ 1.8 were used for RNA-seq. Raw reads were filtered using FastQC software (Babraham Bioinformatics website) to remove poor-quality reads and adapters. Sequences with a Phred score above 20 were considered clean reads. All clean reads were mapped to the *P. ultimum* reference genome, using Hisat2 with the parameters of -min-intronlen 30-max-intronlen 5000-dta (Pertea et al. 2016). SAMtools was used to convert and sort SAM files (Li et al. 2009). Based on the lengths of each gene and the counts of uniquely mapped reads, gene expression levels were normalized as RPKM using the Stringtie tool (Pertea et al. 2016). The RPKM values of PyRXLRs during different stages are shown in Supplementary Table S9. Heat maps and clustering (using hierarchical clustering methods) of gene expression patterns were performed using MeV software (Saeed et al. 2003).

DAB staining.

N. benthamiana leaves with ectopic expression of the indicated RXLR effectors were detached at 48 hpi and were then stained with a 1-mg/ml DAB solution for 8 h in the dark. After destaining with ethanol, leaves were examined under light microscopy. *Phytophthora capsici*-inoculated *N. benthamiana* leaves were stained with a 1-mg/ml DAB solution for 8 h in the dark at 12 hpi and were then destained with ethanol before photographing.

ACKNOWLEDGMENTS

We thank B. Tyler at Oregon State University for his useful suggestions and discussion.

AUTHOR-RECOMMENDED INTERNET RESOURCES

ESpritz: <http://protein.bio.unipd.it/espritz>
 Babraham Bioinformatics FastQC software:
<https://www.bioinformatics.babraham.ac.uk/index.html>
 GitHub permutation code: <https://github.com/Gan-Ai/sequence-permutation>
 The Joint Genome Institute website: <http://genome.jgi-psf.org>
 National Center for Biotechnology Information: <https://www.ncbi.nlm.nih.gov>

LITERATURE CITED

Adhikari, B. N., Hamilton, J. P., Zerillo, M. M., Tisserat, N., Lévesque, C. A., and Buell, C. R. 2013. Comparative genomics reveals insight into virulence strategies of plant pathogenic oomycetes. *PLoS One* 8:e75072.

- Amselem, J., Cuomo, C. A., van Kan, J. A., Viaud, M., Benito, E. P., Couloux, A., Coutinho, P. M., de Vries, R. P., Dyer, P. S., Fillinger, S., Fournier, E., Gout, L., Hahn, M., Kohn, L., Lapalu, N., Plummer, K. M., Pradier, J. M., Quévillon, E., Sharon, A., Simon, A., ten Have, A., Tudzynski, B., Tudzynski, P., Wincker, P., Andrew, M., Anthouard, V., Beever, R. E., Beffa, R., Benoit, I., Bouzid, O., Brault, B., Chen, Z., Choquer, M., Collémare, J., Cotton, P., Danchin, E. G., Da Silva, C., Gautier, A., Giraud, C., Giraud, T., Gonzalez, C., Grossetete, S., Güldener, U., Henrissat, B., Howlett, B. J., Kodira, C., Kretschmer, M., Lappartient, A., Leroy, M., Levis, C., Mauceli, E., Neuvéglise, C., Oeser, B., Pearson, M., Poulain, J., Poussereau, N., Quesneville, H., Rasle, C., Schumacher, J., Séguens, B., Sexton, A., Silva, E., Sirven, C., Soanes, D. M., Talbot, N. J., Templeton, M., Yandava, C., Yarden, O., Zeng, Q., Rollins, J. A., Lebrun, M. H., and Dickman, M. 2011. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet.* 7:e1002230.
- Anderson, R. G., Deb, D., Fedkenheuer, K., and McDowell, J. M. 2015. Recent progress in rxlr effector research. *Mol. Plant-Microbe Interact.* 28:1063-1072.
- Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., Martinez, D., Putnam, N. H., Zhou, S., Allen, A. E., Apt, K. E., Bechner, M., Brzezinski, M. A., Chahal, B. K., Chiovitti, A., Davis, A. K., Demarest, M. S., Deter, J. C., Glavina, T., Goodstein, D., Hadi, M. Z., Hellsten, U., Hildebrand, M., Jenkins, B. D., Jurka, J., Kapitonov, V. V., Kröger, N., Lau, W. W., Lane, T. W., Larimer, F. W., Lippmeier, J. C., Lucas, S., Medina, M., Montsant, A., Obornik, M., Parker, M. S., Palenik, B., Pazour, G. J., Richardson, P. M., Rynearson, T. A., Saito, M. A., Schwartz, D. C., Thamatrakoln, K., Valentin, K., Vardi, A., Wilkerson, F. P., and Rokhsar, D. S. 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. *Science* 306:79-86.
- Baxter, L., Tripathy, S., Ishaque, N., Boot, N., Cabral, A., Kemen, E., Thines, M., Ah-Fong, A., Anderson, R., Badejoko, W., Bittner-Eddy, P., Boore, J. L., Chibucos, M. C., Coates, M., Dehal, P., Delehaunty, K., Dong, S., Downton, P., Dumas, B., Fabro, G., Fronick, C., Fuerstenberg, S. I., Fulton, L., Gaulin, E., Govers, F., Hughes, L., Humphray, S., Jiang, R. H. Y., Judelson, H., Kamoun, S., Kyung, K., Meijer, H., Minx, P., Morris, P., Nelson, J., Phuntumart, V., Qutob, D., Rehmany, A., Rougon-Cardoso, A., Ryden, P., Torto-Alalibo, T., Studholme, D., Wang, Y., Win, J., Wood, J., Clifton, S. W., Rogers, J., Van den Ackerveken, G., Jones, J. D. G., McDowell, J. M., Beynon, J., and Tyler, B. M. 2010. Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. *Science* 330:1549-1551.
- Bendtsen, J. D., Nielsen, H., von Heijne, G., and Brunak, S. 2004. Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* 340:783-795.
- Benhamou, N., le Floch, G., Vallance, J., Gerbore, J., Grizard, D., and Rey, P. 2012. *Pythium oligandrum*: An example of opportunistic success. *Microbiology* 158:2679-2694.
- Benhamou, N., Rey, P., Chérif, M., Hockenhull, J., and Tirilly, Y. 1997. Treatment with the mycoparasite *Pythium oligandrum* triggers induction of defense-related reactions in tomato roots when challenged with *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 87:108-122.
- Blattner, F. R., Plunkett, G., 3rd, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277:1453-1462.
- Bowler, C., Allen, A. E., Badger, J. H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R. P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., Verret, F., Berges, J. A., Brownlee, C., Cadoret, J. P., Chiovitti, A., Choi, C. J., Coesel, S., De Martino, A., Deter, J. C., Durkin, C., Falcatore, A., Fournet, J., Haruta, M., Huysman, M. J., Jenkins, B. D., Jiroutova, K., Jorgensen, R. E., Joubert, Y., Kaplan, A., Kröger, N., Kroth, P. G., La Roche, J., Lindquist, E., Lommer, M., Martin-Jézéquel, V., Lopez, P. J., Lucas, S., Mangogna, M., McGinnis, K., Medlin, L. K., Montsant, A., Oudot-Le Secq, M. P., Napoli, C., Obornik, M., Parker, M. S., Petit, J. L., Porcel, B. M., Poulsen, N., Robison, M., Rychlewski, L., Rynearson, T. A., Schmutz, J., Shapiro, H., Siat, M., Stanley, M., Sussman, M. R., Taylor, A. R., Vardi, A., von Dassow, P., Vyverman, W., Willis, A., Wyrwicz, L. S., Rokhsar, D. S., Weissenbach, J., Armbrust, E. V., Green, B. R., Van de Peer, Y., and Grigoriev, I. V. 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456:239-244.
- C. elegans Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: A platform for investigating biology. *Science* 282:2012-2018.
- Deb, D., Anderson, R. G., How-Yew-Kin, T., Tyler, B. M., and McDowell, J. M. 2018. Conserved RxLR effectors from oomycetes *Hyaloperonospora arabidopsidis* and *Phytophthora sojae* suppress PAMP- and effector-triggered immunity in diverse plants. *Mol. Plant-Microbe Interact.* 31:374-385.
- Dong, S., Raffaele, S., and Kamoun, S. 2015. The two-speed genomes of filamentous pathogens: Waltz with plants. *Curr. Opin. Genet. Dev.* 35: 57-65.
- Dou, D., and Zhou, J. M. 2012. Phytopathogen effectors subverting host immunity: Different foes, similar battleground. *Cell Host Microbe* 12: 484-495.
- Duan, Y., Zhou, L., Hall, D. G., Li, W., Doddapaneni, H., Lin, H., Liu, L., Vahling, C. M., Gabriel, D. W., Williams, K. P., Dickerman, A., Sun, Y., and Gottwald, T. 2009. Complete genome sequence of citrus huanglongbing bacterium, '*Candidatus Liberibacter asiaticus*' obtained through metagenomics. *Mol. Plant-Microbe Interact.* 22:1011-1020.
- Duplessis, S., Cuomo, C. A., Lin, Y. C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D. L., Hacquard, S., Amselem, J., Cantarel, B. L., Chiu, R., Coutinho, P. M., Feau, N., Field, M., Frey, P., Gelhaye, E., Goldberg, J., Grabherr, M. G., Kodira, C. D., Kohler, A., Kües, U., Lindquist, E. A., Lucas, S. M., Mago, R., Mauceli, E., Morin, E., Murat, C., Pangilan, J. L., Park, R., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Salamov, A. A., Schmutz, J., Selles, B., Shapiro, H., Tanguay, P., Tuskan, G. A., Henrissat, B., Van de Peer, Y., Rouzé, P., Ellis, J. G., Dodds, P. N., Schein, J. E., Zhong, S., Hamelin, R. C., Grigoriev, I. V., Szabo, L. J., and Martin, F. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc. Natl. Acad. Sci. U.S.A.* 108: 9166-9171.
- Edgar, R. C. 2004. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113.
- Haas, B. J., Kamoun, S., Zody, M. C., Jiang, R. H., Handsaker, R. E., Cano, L. M., Grabherr, M., Kodira, C. D., Raffaele, S., Torto-Alalibo, T., Bozkurt, T. O., Ah-Fong, A. M., Alvarado, L., Anderson, V. L., Armstrong, M. R., Avrova, A., Baxter, L., Beynon, J., Boevink, P. C., Bollmann, S. R., Bos, J. I., Bulone, V., Cai, G., Cakir, C., Carrington, J. C., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M. A., Fugelstad, J., Gilroy, E. M., Gnerre, S., Green, P. J., Grenville-Briggs, L. J., Griffith, J., Grünwald, N. J., Horn, K., Horner, N. R., Hu, C. H., Huitema, E., Jeong, D. H., Jones, A. M., Jones, J. D., Jones, R. W., Karlsson, E. K., Kunjeti, S. G., Lamour, K., Liu, Z., Ma, L., Maclean, D., Chibucos, M. C., McDonald, H., McWalters, J., Meijer, H. J., Morgan, W., Morris, P. F., Munro, C. A., O'Neill, K., Ospina-Giraldo, M., Pinzón, A., Pritchard, L., Ramsahoye, B., Ren, Q., Restrepo, S., Roy, S., Sadanandom, A., Savidor, A., Schornack, S., Schwartz, D. C., Schumann, U. D., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D. J., Sykes, S., Thines, M., van de Vondervoort, P. J., Phuntumart, V., Wawra, S., Weide, R., Win, J., Young, C., Zhou, S., Fry, W., Meyers, B. C., van West, P., Ristaino, J., Govers, F., Birch, P. R., Whisson, S. C., Judelson, H. S., and Nusbaum, C. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461:393-398.
- Jacq, C., Alt-Mörbe, J., Andre, B., Arnold, W., Bahr, A., Ballesta, J. P., Barges, M., Baron, L., Becker, A., Biteau, N., Blöcker, H., Blugeon, C., Boskovic, J., Brandt, P., Brückner, M., Buitrago, M. J., Coster, F., Delaveau, T., del Rey, F., Dujon, B., Eide, L. G., Garcia-Cantalejo, J. M., Goffeau, A., Gomez-Peris, A., Zaccaria, P. et al. 1997. The nucleotide sequence of *Saccharomyces cerevisiae* chromosome IV. *Nature* 387: 75-78.
- Jiang, R. H., de Bruijn, I., Haas, B. J., Belmonte, R., Löbach, L., Christie, J., van den Ackerveken, G., Bottin, A., Bulone, V., Díaz-Moreno, S. M., Dumas, B., Fan, L., Gaulin, E., Govers, F., Grenville-Briggs, L. J., Horner, N. R., Levin, J. Z., Mammella, M., Meijer, H. J., Morris, P., Nusbaum, C., Oome, S., Phillips, A. J., van Rooyen, D., Rzeszutek, E., Saraiva, M., Secombes, C. J., Seidl, M. F., Snel, B., Stassen, J. H., Sykes, S., Tripathy, S., van den Berg, H., Vega-Arreguin, J. C., Wawra, S., Young, S. K., Zeng, Q., Dieguez-Urbeondo, J., Russ, C., Tyler, B. M., and van West, P. 2013. Distinctive expansion of potential virulence genes in the genome of the oomycete fish pathogen *Saprolegnia parasitica*. *PLoS Genet.* 9:e1003272.
- Jiang, R. H., Tripathy, S., Govers, F., and Tyler, B. M. 2008. RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members. *Proc. Natl. Acad. Sci. U.S.A.* 105:4874-4879.
- Jones, J. D., and Dangl, J. L. 2006. The plant immune system. *Nature* 444: 323-329.
- Kale, S. D., Gu, B., Capelluto, D. G., Dou, D., Feldman, E., Rumore, A., Arredondo, F. D., Hanlon, R., Fudal, I., Rouxel, T., Lawrence, C. B., Shan, W., and Tyler, B. M. 2010. External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell* 142: 284-295.

- Kamoun, S., Furzer, O., Jones, J. D., Judelson, H. S., Ali, G. S., Dalio, R. J., Roy, S. G., Schena, L., Zambounis, A., Panabieres, F., Cahill, D., Ruocco, M., Figueiredo, A., Chen, X. R., Hulvey, J., Stam, R., Lamour, K., Gijzen, M., Tyler, B. M., Grünwald, N. J., Mukhtar, M. S., Tomé, D. F., Tör, M., Van Den Ackerveken, G., McDowell, J., Daayf, F., Fry, W. E., Lindqvist-Kreuzer, H., Meijer, H. J., Petre, B., Ristaino, J., Yoshida, K., Birch, P. R., and Govers, F. 2015. The top 10 oomycete pathogens in molecular plant pathology. *Mol. Plant Pathol.* 16: 413-434.
- Kämper, J., Kahmann, R., Bölker, M., Ma, L. J., Brefort, T., Saville, B. J., Banuett, F., Kronstad, J. W., Gold, S. E., Müller, O., Perlin, M. H., Wösten, H. A., de Vries, R., Ruiz-Herrera, J., Reynaga-Peña, C. G., Snetselaar, K., McCann, M., Pérez-Martín, J., Feldbrügge, M., Basse, C. W., Steinberg, G., Ibeas, J. I., Holloman, W., Guzman, P., Farman, M., Stajich, J. E., Sentandreu, R., González-Prieto, J. M., Kennell, J. C., Molina, L., Schirawski, J., Mendoza-Mendoza, A., Greilinger, D., Münch, K., Rössel, N., Scherer, M., Vranes, M., Ladendorf, O., Vincon, V., Fuchs, U., Sandrock, B., Meng, S., Ho, E. C., Cahill, M. J., Boyce, K. J., Klose, J., Klosterman, S. J., Deelstra, H. J., Ortiz-Castellanos, L., Li, W., Sanchez-Alonso, P., Schreier, P. H., Häuser-Hahn, I., Vaupel, M., Koopmann, E., Friedrich, G., Voss, H., Schlüter, T., Margolis, J., Platt, D., Swimmer, C., Gnirke, A., Chen, F., Vysotskaia, V., Mannhaupt, G., Güldener, U., Münsterkötter, M., Haase, D., Oesterheld, M., Mewes, H. W., Mauceli, E. W., DeCaprio, D., Wade, C. M., Butler, J., Young, S., Jaffe, D. B., Calvo, S., Nusbaum, C., Galagan, J., and Birren, B. W. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444:97-101.
- Kanneganti, T. D., Huitema, E., Cakir, C., and Kamoun, S. 2006. Synergistic interactions of the plant cell death pathways induced by *Phytophthora infestans* Nep1-like protein PiNPP1.1 and INF1 elicitor. *Mol. Plant-Microbe Interact.* 19:854-863.
- Kemen, E., Gardiner, A., Schultz-Larsen, T., Kemen, A. C., Balmuth, A. L., Robert-Seilaniantz, A., Bailey, K., Holub, E., Studholme, D. J., Maclean, D., and Jones, J. D. 2011. Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*. *PLoS Biol.* 9:e1001094.
- Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. 2001. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J. Mol. Biol.* 305:567-580.
- Kushwaha, S. K., Vetukuri, R. R., and Grenville-Briggs, L. J. 2017. Draft genome sequence of the mycoparasitic oomycete *Pythium oligandrum* strain CBS 530.74. *Genome Announc.* 5:e00346-17.
- Latijnhouwers, M., de Wit, P. J., and Govers, F. 2003. Oomycetes and fungi: Similar weaponry to attack plants. *Trends Microbiol.* 11:462-469.
- Lévesque, C. A., Brouwer, H., Cano, L., Hamilton, J. P., Holt, C., Huitema, E., Raffaele, S., Robideau, G. P., Thines, M., Win, J., Zerillo, M. M., Beakes, G. W., Boore, J. L., Busam, D., Dumas, B., Ferriera, S., Fuerstenberg, S. I., Gachon, C. M., Gaulin, E., Govers, F., Grenville-Briggs, L., Horner, N., Hostetler, J., Jiang, R. H., Johnson, J., Krjaejun, T., Lin, H., Meijer, H. J., Moore, B., Morris, P., Phuntmart, V., Puui, D., Shetty, J., Stajich, J. E., Tripathy, S., Wawra, S., van West, P., Whitty, B. R., Coutinho, P. M., Henrissat, B., Martin, F., Thomas, P. D., Tyler, B. M., De Vries, R. P., Kamoun, S., Yandell, M., Tisserat, N., and Buell, C. R. 2010. Genome sequence of the necrotrophic plant pathogen *Pythium ultimum* reveals original pathogenicity mechanisms and effector repertoire. *Genome Biol.* 11:R73.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., and 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078-2079.
- Li, Q., Ai, G., Shen, D., Zou, F., Wang, J., Bai, T., Chen, Y., Li, S., Zhang, M., Jing, M., and Dou, D. 2019. A *Phytophthora capsici* effector targets ACD11 binding partners that regulate ROS-mediated defense response in *Arabidopsis*. *Mol. Plant* 12:565-581.
- Links, M. G., Holub, E., Jiang, R. H., Sharpe, A. G., Hegedus, D., Beynon, E., Sillito, D., Clarke, W. E., Uzuhashi, S., and Borhan, M. H. 2011. De novo sequence assembly of *Albugo candida* reveals a small genome relative to other biotrophic oomycetes. *BMC Genomics* 12:503.
- McCarthy, C. G. P., and Fitzpatrick, D. A. 2017. Phylogenomic reconstruction of the oomycete phylogeny derived from 37 genomes. *MSphere* 2:e00095-17.
- Monaghan, J., and Zipfel, C. 2012. Plant pattern recognition receptor complexes at the plasma membrane. *Curr. Opin. Plant Biol.* 15:349-357.
- Nguyen, L. T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* 32:268-274.
- Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., Graham, S., Houfek, T. D., Liu, Q., Mitros, T., Schaff, J., Schaffer, R., Scholl, E., Sosinski, B. R., Thomas, V. P., and Windham, E. 2008. Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proc. Natl. Acad. Sci. U.S.A.* 105:14802-14807.
- Paul, B. 1999. *Pythium periplocum*, an aggressive mycoparasite of *Botrytis cinerea* causing the gray mould disease of grape-vine. *FEMS Microbiol. Lett.* 181:277-280.
- Pertea, M., Kim, D., Pertea, G. M., Leek, J. T., and Salzberg, S. L. 2016. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.* 11:1650-1667.
- Qi, J., Wang, J., Gong, Z., and Zhou, J. M. 2017. Apoplastic ROS signaling in plant immunity. *Curr. Opin. Plant Biol.* 38:92-100.
- Raffaele, S., Farrer, R. A., Cano, L. M., Studholme, D. J., MacLean, D., Thines, M., Jiang, R. H., Zody, M. C., Kunjeti, S. G., Donofrio, N. M., Meyers, B. C., Nusbaum, C., and Kamoun, S. 2010. Genome evolution following host jumps in the Irish potato famine pathogen lineage. *Science* 330:1540-1543.
- Raffaele, S., and Kamoun, S. 2012. Genome evolution in filamentous plant pathogens: Why bigger can be better. *Nat. Rev. Microbiol.* 10: 417-430.
- Rujirawat, T., Patumcharoenpol, P., Lohnoo, T., Yingyong, W., Kumsang, Y., Payattikul, P., Tangphatsornruang, S., Suriyaphol, P., Reamtoong, O., Garg, G., Kittichotirat, W., and Krajaeun, T. 2018. Probing the phylogenomics and putative pathogenicity genes of *Pythium insidiosum* by oomycete genome analyses. *Sci. Rep.* 8:4135.
- Saeed, A. I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturm, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V., and Quackenbush, J. 2003. TM4: A free, open-source system for microarray data management and analysis. *Biotechniques* 34:374-378.
- Shen, D., Li, Q., Sun, P., Zhang, M., and Dou, D. 2017. Intrinsic disorder is a common structural characteristic of RxLR effectors in oomycete pathogens. *Fungal Biol.* 121:911-919.
- Shen, D., Tang, Z., Wang, C., Wang, J., Dong, Y., Chen, Y., Wei, Y., Cheng, B., Zhang, M., Grenville-Briggs, L. J., Tyler, B. M., Dou, D., and Xia, A. 2019. Infection mechanisms and putative effector repertoire of the mosquito pathogenic oomycete *Pythium guiyangense* uncovered by genomic analysis. *PLoS Genet.* 15:e1008116.
- Smits, T. H., Rezzonico, F., Kamber, T., Blom, J., Goesmann, A., Frey, J. E., and Duffy, B. 2010. Complete genome sequence of the fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Mol. Plant-Microbe Interact.* 23:384-393.
- Song, T., Ma, Z., Shen, D., Li, Q., Li, W., Su, L., Ye, T., Zhang, M., Wang, Y., and Dou, D. 2015. An oomycete CRN effector reprograms expression of plant HSP genes by targeting their promoters. *PLoS Pathog.* 11:e1005348.
- Subramanian, B., Gao, S., Lercher, M. J., Hu, S., and Chen, W. H. 2019. Evolveview v3: A webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids Res.* 47 (W1):W270-W275.
- Takenaka, S., Nishio, Z., and Nakamura, Y. 2003. Induction of defense reactions in sugar beet and wheat by treatment with cell wall protein fractions from the mycoparasite *Pythium oligandrum*. *Phytopathology* 93:1228-1232.
- Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H., Aerts, A., Arredondo, F. D., Baxter, L., Bensasson, D., Beynon, J. L., Chapman, J., Damasceno, C. M., Dorrance, A. E., Dou, D., Dickerman, A. W., Dubchak, I. L., Garbelotto, M., Gijzen, M., Gordon, S. G., Govers, F., Grünwald, N. J., Huang, W., Ivors, K. L., Jones, R. W., Kamoun, S., Krampis, K., Lamour, K. H., Lee, M. K., McDonald, W. H., Medina, M., Meijer, H. J., Nordberg, E. K., Maclean, D. J., Ospina-Giraldo, M. D., Morris, P. F., Phuntmart, V., Putnam, N. H., Rash, S., Rose, J. K., Sakihama, Y., Salamov, A. A., Savidor, A., Scheuring, C. F., Smith, B. M., Sobral, B. W., Terry, A., Torto-Alalibo, T. A., Win, J., Xu, Z., Zhang, H., Grigoriev, I. V., Rokhsar, D. S., and Boore, J. L. 2006. *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313:1261-1266.
- Van Kan, J. A., Stassen, J. H., Mosbach, A., Van Der Lee, T. A., Faino, L., Farmer, A. D., Papasotiropoulos, D. G., Zhou, S., Seidl, M. F., Cottam, E., Edel, D., Hahn, M., Schwartz, D. C., Dietrich, R. A., Widdison, S., and Scalliet, G. 2017. A gapless genome sequence of the fungus *Botrytis cinerea*. *Mol. Plant Pathol.* 18:75-89.
- Walsh, I., Martin, A. J., Di Domenico, T., and Tosatto, S. C. 2012. ESpritz: Accurate and fast prediction of protein disorder. *Bioinformatics* 28: 503-509.
- Wang, Q., Han, C., Ferreira, A. O., Yu, X., Ye, W., Tripathy, S., Kale, S. D., Gu, B., Sheng, Y., Sui, Y., Wang, X., Zhang, Z., Cheng, B., Dong, S., Shan, W., Zheng, X., Dou, D., Tyler, B. M., and Wang, Y. 2011.

- Transcriptional programming and functional interactions within the *Phytophthora sojae* RXLR effector repertoire. *Plant Cell* 23:2064-2086.
- Wang, Y., Tang, H., Debarry, J. D., Tan, X., Li, J., Wang, X., Lee, T. H., Jin, H., Marler, B., Guo, H., Kissinger, J. C., and Paterson, A. H. 2012. MCSanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* 40:e49.
- Wawra, S., Bain, J., Durward, E., de Bruijn, I., Minor, K. L., Matena, A., Löbach, L., Whisson, S. C., Bayer, P., Porter, A. J., Birch, P. R., Secombes, C. J., and van West, P. 2012. Host-targeting protein 1 (SpHtp1) from the oomycete *Saprolegnia parasitica* translocates specifically into fish cells in a tyrosine-O-sulphate-dependent manner. *Proc. Natl. Acad. Sci. U.S.A.* 109:2096-2101.
- Whisson, S. C., Boevink, P. C., Moleleki, L., Avrova, A. O., Morales, J. G., Gilroy, E. M., Armstrong, M. R., Grouffaud, S., van West, P., Chapman, S., Hein, I., Toth, I. K., Pritchard, L., and Birch, P. R. 2007. A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature* 450:115-118.
- Win, J., Morgan, W., Bos, J., Krasileva, K. V., Cano, L. M., Chaparro-Garcia, A., Ammar, R., Staskawicz, B. J., and Kamoun, S. 2007. Adaptive evolution has targeted the C-terminal domain of the RXLR effectors of plant pathogenic oomycetes. *Plant Cell* 19:2349-2369.
- Yin, L., An, Y., Qu, J., Li, X., Zhang, Y., Dry, I., Wu, H., and Lu, J. 2017. Genome sequence of *Plasmopara viticola* and insight into the pathogenic mechanism. *Sci. Rep.* 7:46553.
- Zheng, X., McLellan, H., Fraiture, M., Liu, X., Boevink, P. C., Gilroy, E. M., Chen, Y., Kandel, K., Sessa, G., Birch, P. R., and Brunner, F. 2014. Functionally redundant RXLR effectors from *Phytophthora infestans* act at different steps to suppress early flg22-triggered immunity. *PLoS Pathog.* 10:e1004057.