Draft genome sequencing and secretome analysis of fungal phytopathogen *Ascochyta rabiei* **provides insight into the necrotrophic effector repertoire**

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Supplementary Figure 1. Determination of the mating type of *A. rabiei* **D2.** The mating type was determined by PCR using *MAT* idiomorph-specific primers. **(a)** and **(b)** A schematic representation of the structural organization of *A. rabiei MAT1-1* and *MAT1-2*. **(c)** An ethidium bromide-stained 1% agarose gel showing bands obtained by PCR to detect the *MAT* locus. The lanes numbered 1 and 2 represent PCR with primer pairs Com1 (common flanking primer)/SP21 (*MAT1-1*-specific) and Com1/Tail 5 (*MAT1-2*-specific), respectively. The Ar628 strain of *A. rabiei* was used as a control for mating type 1.

Supplementary Figure 2. Gene Ontology (GO) functional annotations of predicted *A. rabiei* **genes.** GO classification was performed based on similarity searches against the NCBI non-redundant database using BLAST2GO software. **(a)** The genes were enriched into three primary GO categories. In total, 4,833 genes were assigned GO terms in the biological process category; however, 4,626 and 2,282 genes were assigned GO terms in the molecular function and cellular component categories, respectively. **(b)** Different terms under each GO category (biological process, molecular function and cellular component) are displayed. The major 10 categories in biological process, 12 categories in molecular function, and 10 categories in cellular component are shown. The *x*-axis represents the number of genes in a functional group, and the *y*-axis represents the GO terms.

Supplementary Figure 3. The *A. rabiei* **predicted genes assigned with Gene Ontology (GO) functional annotations.** BLAST2GO software was used for GO classification based on similarity searches against the NCBI non-redundant database. In total, 5,511 genes (52% of total genes) were assigned GO terms in three primary categories. The Venn diagram shows *A. rabiei* genes having unique and shared GO terms. Almost 1,511 genes were assigned GO terms in all three categories, i.e., molecular function, biological process and cellular component. However, 439, 159 and 194 genes had GO terms unique to molecular function, biological process and cellular component, respectively.

Supplementary Figure 4. Functional annotations of predicted *A. rabiei* **proteins based on KEGG Orthology.** Annotation was performed using the KEGG database and blastKOALA. The proteins were functionally categorized into 10 distinct categories.

Supplementary Figure 5. ORF length distribution of the predicted genes of *A. rabiei***.** The ORFs of lengths between 801-1000 bp were found to be the most abundant, followed by the ORFs of lengths between 1001-1200 bp.

Supplementary Figure 6. Relative abundance of transposable elements in the *A. rabiei* **genome.** The proportion (%) of different types of repetitive sequences present in the *A. rabiei* genome. In the LTR type retrotransposons, *Gypsy* is the most abundant type of TE, followed by *Copia*. However, the non-LTR Tad1 is the least abundant form of TE. Repetitive elements were identified *de novo* using RepeatScout and then annotated manually by a TBLASTX search against RepBase.

Supplementary Figure 7. RIP mutation in *Gypsy* **class of LTR retrotransposons shown as RIPCAL output.** The upper panel of output shows multiple alignment of the genomic regions corresponding to repeat units of each class. Polymorphic nucleotides are colored according to the type of RIP mutation detected. Black: invariant nucleotide; red: $CpA \leftrightarrow TpA$ or $TpG \leftrightarrow TpA$ mutations; dark blue: $CpC \leftrightarrow TpC$ or $GpG \leftrightarrow GpA$ mutations; green: CpG \leftrightarrow TpG or CpG \leftrightarrow CpA mutations; turquoise blue: CpT \leftrightarrow TpT or ApG \leftrightarrow ApA mutations. The lower panel of each output shows the frequency plot of the RIP mutations in accordance with the multiple alignment shown above over a rolling sequence window. Nucleotide polymorphisms are color-coded as in the upper panel.

Supplementary Figure 8. RIP mutation in *Copia* **class of LTR retrotransposons shown as RIPCAL output.** The upper panel of output shows multiple alignment of the genomic regions corresponding to repeat units of each class. Polymorphic nucleotides are colored according to the type of RIP mutation detected. The lower panel of each output shows the frequency plot of the RIP mutations in accordance with the multiple alignment shown above over a rolling sequence window. Nucleotide polymorphisms are color-coded as in the upper panel.

Supplementary Figure 9. RIP mutation in Tad1 class of non-LTR retrotransposons shown as RIPCAL output.

Supplementary Figure 10. RIP mutation in Tcl-Mariner class of DNA transposons shown as RIPCAL output.

Supplementary Figure 11. RIP mutation in unclassified transposable elements shown as RIPCAL output. RIP mutation is shown for the unclassified repeat sequences.

Supplementary Figure 12. SSRs across the genome of *A. rabiei***. (a)** The total numbers of mono-, di-, tri-, tetra-, penta- and hexa-nucleotide SSRs present in the genome are shown.

Tri-nucleotide SSRs are the most dominant type of SSRs, whereas penta-nucleotide SSRs are least abundant. The total number of each SSR is shown in parenthesis. The SSRs were identified in the genome using MIcroSAtellite identification tool (MISA). **(b)** Relative abundance of SSRs across the *A. rabiei* genome is shown. Abundance is the total number of SSRs present per Mb of sequence analyzed. The tri-nucleotide repeat showed the highest relative abundance, while the penta-nucleotide repeat showed the lowest relative abundance. **(c)** Relative density of SSRs is shown. Density is the total sequence length (bp) contributed by each SSR per Mb of DNA of the analyzed sequence. The tri-nucleotide repeat showed the highest relative density, whereas the tetra-nucleotide repeat showed the lowest relative density.

Supplementary Figure 13. The GHs present in gene families unique to *A. rabiei***.** The bars represent the number of distinct GHs present in the 693 gene families that are unique to *A. rabiei* when compared to other closely related necrotrophic fungi i.e. *C. heterostrophus*, *P. tritici-repentis* and *S. nodorum*. The enzyme classes are indicated by codes that are defined by the CAZyme database.

Superfamilies of transporters

Supplementary Figure 14. Transporters identified in the *A. rabiei* **genome.** The identification and classification of transporters were performed based on similarity searches against the Transporter Classification Database (TCDB). The identified transporters were classified into seven distinct superfamilies, i.e., channels/pores, electrochemical potentialdriven transporters, primary active transporters, group translocators, transmembrane electron carriers, accessory electron carriers and incompletely characterized transporters. The most abundant transporters belonged to the electrochemical potential-driven superfamily, followed by primary active transporters.

OCBM OCE OGH OGT OPL OAA

 $\mathbf b$

Polysaccharide Lyases

30%

Carbohydrate Esterases

■GT48 ■GT2 ■GT34 ■GT4 ■GT41 ■Others

Glycosyl Transferases

■AA3 ■AA7 ■AA9 ■AA6 ■AA1 ■Others

Auxiliary Activities

a

Supplementary Figure 15. Carbohydrate-active enzyme (CAZyme) functional annotations of *A. rabiei* **predicted genes. (a)** Summary displayed for the six CAZyme categories: carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), glucoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs) and auxiliary activities (AAs). **(b)** Distinct summaries of each of the CAZyme category representing the most abundant CAZyme classes. The prediction of CAZymes from the predicted genes of *A. rabiei* and their classification were performed using tools from the Carbohydrate-Active EnZymes (CAZyme) database.

Ascochyta rabiei GH₁ Blumeria graminis f.sp. tritici GH₃ GH₅ GH₆ GH7 GH₉ GH45 40 20 $\pmb{0}$ 10 20 30 40 30 10 $\pmb{0}$ Cochliobolus heterostrophus GH₁ Blumeria graminis f.sp. hordei GH₃ GH₅ GH₆ GH7 GH₉ GH45 40 30 20 10 $\mathbf 0$ $\overline{0}$ 10 20 30 40 Pyrenophora tritici-repentis GH₁ Claviceps purpurea GH₃ GH₅ GH₆ GH7 GH₉ GH45 40 20 30 10 $\pmb{0}$ 10 20 $\mathsf 0$ 30 40 Number of genes Number of genes

Biotrophic fungi

Necrotrophic fungi

Supplementary Figure 16. Diversity of genes encoding CAZymes involved in cellulose degradation in *A. rabiei***, related necrotrophic fungi and biotrophic fungi.** The bars are representing the number of cellulose degrading enzymes present in the genomes of six fungi.

On the left side, the necrotrophic fungi: *A. rabiei*, *C. heterostrophus* and *P. tritici-repentis* are shown. Whereas on right side, the biotrophic fungi: *Blumeria graminis* f.sp. *tritici*, *Blumeria graminis* f.sp. *hordei* and *Claviceps purpurea* are presented. The enzyme classes are indicated by codes that are defined by the CAZyme database.

Necrotrophic fungi

Biotrophic fungi

Supplementary Figure 17. Diversity of genes encoding CAZymes involved in hemicellulose degradation in *A. rabiei***, related necrotrophic fungi and biotrophic fungi.** The bars are representing the number of hemicellulose degrading enzymes present in the genomes of six fungi. On the left side, the necrotrophic fungi: *A. rabiei*, *C. heterostrophus* and *P. tritici-repentis* are shown. Whereas on right side, the biotrophic fungi: *Blumeria graminis* f.sp. *tritici*, *Blumeria graminis* f.sp. *hordei* and *Claviceps purpurea* are presented. The enzyme classes are indicated by codes that are defined by the CAZyme database.

Supplementary Figure 18. Classification of *A. rabiei* **genes based on the Pathogen-Host Interaction database (PHI-base).** The orthologs of the PHI-base genes were predicted in *A. rabiei* using BLASTP search. Different mutant phenotypic categories of the PHI-base orthologs are shown. Almost 38% of the orthologs exhibited reduced virulence as their mutant phenotype, and 8% of mutants showed complete loss of pathogenicity.

Supplementary Figure 19. Gene Ontology functional annotations of *A. rabiei* **genes predicted to involve in pathogenicity by PHI-base.** GO classification was performed for the genes that were predicted by PHI-base to function in pathogenicity. Classification was performed based on similarity searches against the NCBI non-redundant database using BLAST2GO software. In total, 1,002 genes were assigned GO terms in the biological process category; however, 976 and 575 genes were assigned GO terms in the molecular function and cellular component categories, respectively.

Figure 20. Overview of the computational pipeline used to identify the secretome of *A.*

rabiei. Two approaches were used simultaneously to predict both the classical and nonclassical secreted effector proteins of *A. rabiei*. In total, 758 proteins were predicted in the secretome of *A. rabiei*.

Supplementary Figure 21. Gene Ontology functional annotation of the *A. rabiei* **predicted secretome.** GO terms were assigned to the protein-coding genes of the *A. rabiei* secretome based on similarity searches against the NCBI non-redundant database using BLAST2GO software. **(a)** Based on significant GO terms, the genes were enriched into three primary GO categories: biological process, molecular function and cellular component. In total, 334 genes were assigned GO terms in the molecular function category; however, 321 and 71 genes were assigned GO terms in the biological process and cellular component categories, respectively. **(b)** Different terms under each GO category are displayed. The major 8 categories in biological process and 7 categories each in molecular function and cellular component are shown. The *x*-axis represents the number of genes in a functional group, and *y*-axis represents the GO terms.

Supplementary Figure 22. Gene Ontology functional annotation of the protein-coding genes of the *A. rabiei* **secretome.** The GO classification was performed based on similarity searches against the NCBI non-redundant database using BLAST2GO software. In total, 354 genes (46.7% of secretome) were assigned GO terms in three primary categories. The Venn diagram shows protein-coding genes of the *A. rabiei* secretome with unique and shared GO terms. Approximately 55 genes were assigned GO terms in all three categories, i.e., molecular function, biological process and cellular component. However, only 19, 6 and 12 genes had GO terms unique to molecular function, biological process and cellular component, respectively. In total, 258 genes shared GO terms in molecular function and biological process categories.

Supplementary Figure 23. Classification of the *A. rabiei* **secretome based on the Pathogen-Host Interaction database (PHI-base).** The orthologs of the PHI-base genes were predicted in the *A. rabiei* secretome using BLASTP search. Different mutant phenotypic categories of the PHI-base orthologs are shown. Approximately 57% of the identified orthologs showed mutant phenotypes of unaffected pathogenicity. However, almost 32% of the orthologs exhibited reduced virulence as their mutant phenotype, and 7% mutants showed complete loss of pathogenicity.

Supplementary Table 1. Description of libraries prepared for *A. rabiei* genome sequencing.

Supplementary Table 2. Description of *A. rabiei* genome assembly.

Supplementary Table 3. The *A. rabiei* predicted genes indicating the hits obtained from various public databases.

The numbers in the parentheses shows the percentages of the genes having significant hits in the respective databases (E-value $<$ 1e-5).

Supplementary Table 4. The tRNA genes identified in *A. rabiei* along with their respective anticodons.

Transfer RNAs were predicted using tRNAscan-SE with standard settings.

Supplementary Table 5. Classification of transposable elements by TEclass.

Supplementary Table 6. Summary of transposable elements of *A. rabiei*.

Supplementary Table 7. Essential components of gene silencing machinery identified in the genome of *A. rabiei* orthologous to *Neurospora crassa* [97].

^a GenBank accession number of *N. crassa* genes.

b ID of orthologous of *N. crassa* genes in *A. rabiei*.

Abbreviations: RIP, Repeat Induced Point mutation; QDE, Quelling-Defective; MSUD, Meiotic Silencing of Unpaired DNA; SAD, Suppressor of Ascus-Dominance.

Supplementary Table 8. MISA results in the genome survey.

Supplementary Table 9. Number of SSRs and length occupied by them in the genome.

Supplementary Table 10. Number of compound SSRs in the genome.

Supplementary Table 11. Most frequent SSR motifs in the genome. The repeat frequency are shown in closed brackets.

Supplementary Table 12. List of GHs unique in *A. rabiei* among the selected most closely related *Dothideomycetes* fungi.

Supplementary Table 13. Summary counts of CAZymes content present in 1,458 shared orthologous genes families among the necrotrophic fungi *A. rabiei*, *C. heterostrophus* and *P. tritici-repentis*.

Supplementary Table 14. Summary counts of CAZymes content present in 112 shared orthologous genes families among the biotrophic fungi *C. purpurea*, *B. graminis* f.sp. *tritici* and *B. graminis* f.sp. *hordei*.

Supplementary Table 15. Genes encoding transporters in *A. rabiei*.

Supplementary Table 16. Genes involved in putative biosynthetic gene clusters in *A. rabiei*.

Supplementary Table 17. Summary counts of CAZymes content of *A. rabiei*.

Supplementary Table 18. Summary of proteins putatively involved in pathogen-host interactions in *A. rabiei*.

Supplementary Table 19. Summary counts of CAZymes content of *A. rabiei* secretome.

Supplementary Table 20. The effector proteins putatively involved in pathogen-host interactions in *A. rabiei*.

Supplementary Table 21. The unannotated secretory proteins having tandem repeats.

Supplementary Table 22. The prediction of *in planta* localization of mature effector proteins.

Supplementary Table 23. The prediction of NLS in effectors predicted to localize in nucleus inside the host.

Supplementary Table 24. The list of primers used in this study.

