Global pangenome analysis of Pyrenophora tritici-repentis reveals high plasticity and translocation of the ToxA gene between different chromosomes

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Introduction

The necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr) is a major foliar wheat pathogen with annual global losses estimated at ~4.3%[1] (Savary et al., 2019). The Ptr-wheat interaction is used as a model system to help understand the evolution of virulence in necrotrophs. In this work, we sequenced a global collection of 40 Ptr isolates with representatives from each of the eight established races and a novel phenotype recently identified in North Africa[2] (Kamel et al., 2019). Ptr races are defined by their ability to produce different combinations of three effectors (host-selective toxins): ToxA, ToxB, and ToxC. Additionally, two isolates were sequenced with long-reads and annotated to near chromosomal level. These were compared to previously sequenced Ptr isolates (Pt-1C-BFP and M4) to infer chromosomal rearrangements and structural organizations, particularly around effectors

Materials and Methods

Fungal isolates, sequencing, assembly, & annotation

Ptr isolates in this study were collected from Canada (21), Algeria (3), Azerbaijan (8), Syria (3), and Tunisia (5) (Table 1). DNA for Illumina HiSeq X (all isolates, 150bp paired-end) and PacBio RS II sequencing was extracted using 'Genomic-tip 100/G Kit' (Qiagen). Sequencing was performed by Genome Quebec. Illumina reads were assembled with Shovill/SPAdes[3,4] and Pacbio reads assembled with Flye[5] and polished with Pilon[6]. Genomes were annotated with FunGAP[7] (genes) and EDTA[8] (transposons).

Table 1. Races of Ptr and number sequenced (+ = present; - = absent)

Race	PtrTox			Number sequenced
	A	B	C	
1	+	-	+	10
2	+	-	-	6
3	-	-	+	4
4	-	-	-	3
5	-	+	-	8
6	-	+	+	3
7	+	+	-	2
8	+	+	+	3
novel	-	+	-	1
Total				40

Pangenome, phylogeny, & ToxA translocation

Pangloss[9] was used to perform pangenome analysis using the default settings. We included a reference genome of a race 1 isolate (Pt-1C-BFP) retrieved from GenBank. The binary matchtable output was used to generate figures with R. An alignment of all concatenated core-genes was used as input for RAxML[10] to generate a maximum likelihood tree and gene prence/absence used for Hierarchical sets[11]. Syntenic blocks of the *ToxA* containing chromosomes of I73-1 and Pt-1C-BFP were aligned and visualized with Circos[12].

Results

Pangenome

The average genome size was 34.8 ± 2.1 Mb with 13,071 predicted genes. Approximately 43% of genes are conserved across all isolates (core). The remaining 57% of genes were present in a subset of isolates, with 56% of these accessory genes present as singletons (Figure 1a). Most CAZymes (cell wall degrading enzymes) were present in all isolates while the majority of effectors were accessory genes. The high accessory count and continually increasing gene count per genome added (Figure 1b) indicate Ptr has an open pangenome.

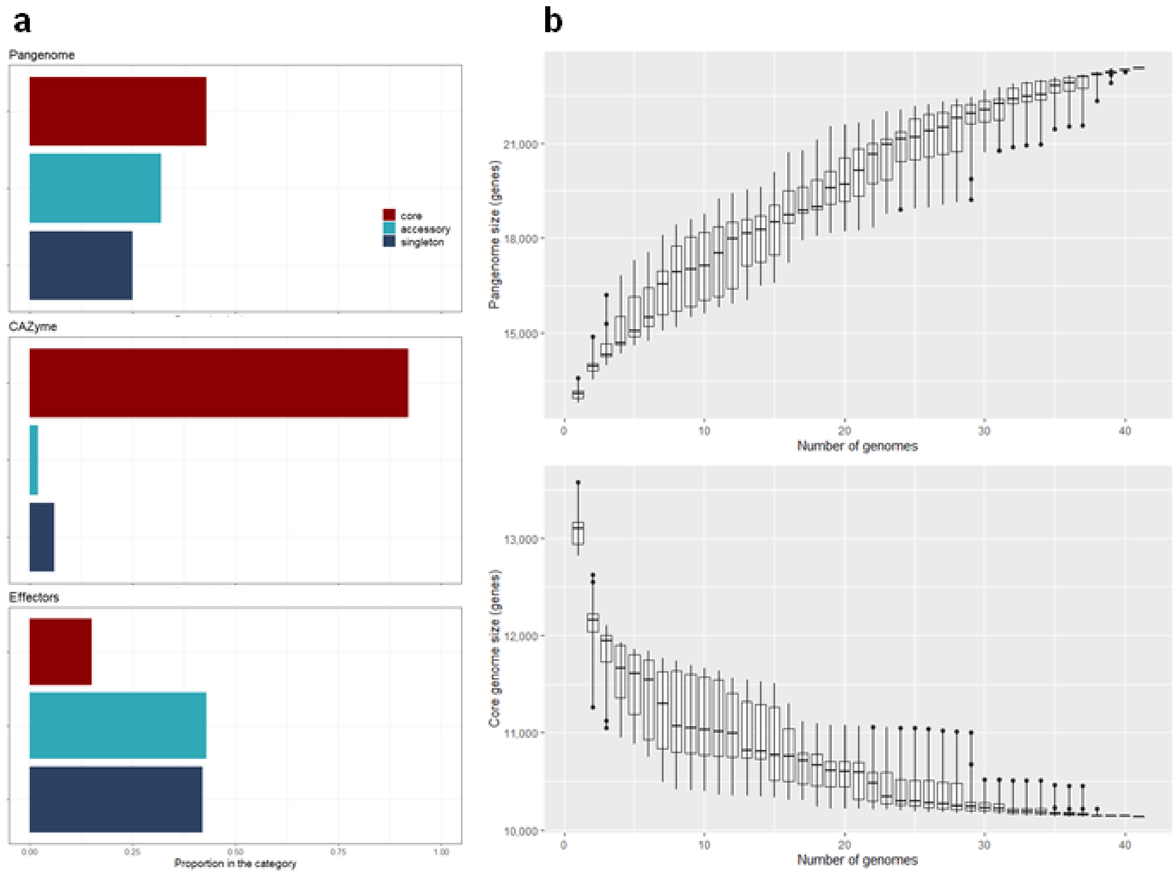

Figure 1. The pangenome of *Pyrenophora tritici-repentis*; a. presence of genes (top), CAZymes (mid), and putative effectors (bottom) in the core (red), accessory (light blue), and singleton (dark blue) sets; b. number of unique genes in the pangenome (top) and core genome (bottom) as genomes are added.

Results

Core phylogeny and hierarchical sets of accessory genes

The maximum-likelihood tree of all core genes produced three major branches (Figure 2). The first contained 20 isolates and is dominated by Canadian ToxA producing isolates. The few isolates not from Canada in this branch produce ToxB. The second branch contains 19 isolates, most of which do no produce ToxA and are from the Fertile Crecent and nearby regions. The final branch, which acts as an outgroup, contains non-pathogenic (race 4) isolates and a weakly virulent race 5. The hierarchical set construction of accessory genes (Figure 2; bordered inset) showed a similar evolutionary structure based solely on gene gains and losses.

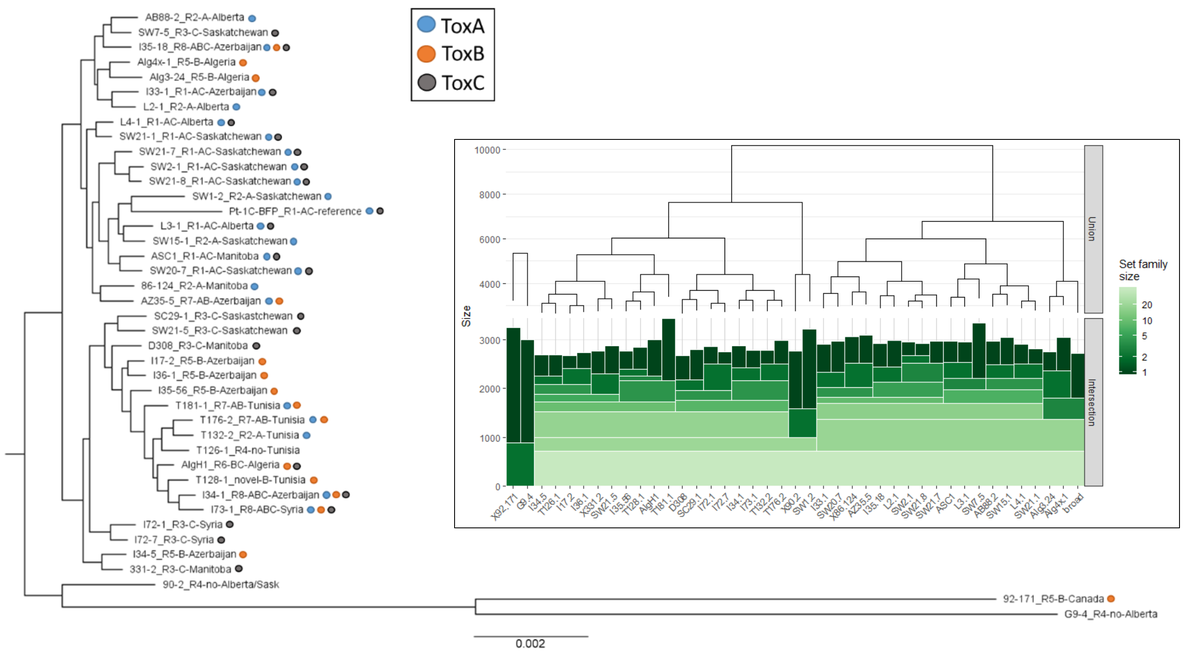

Figure 2. Maximum-likelihood phylogenetic tree of *Pyrenophora-tritici repentis* based on aligned and concatenated genes present in the core genome (total 10,159 genes); bordered inset: hierarchical set of accessory genes, size represents number of genes shared by isolates in each cluster, where inclusion in a cluster is based on any given isolate overlapping with the horizontal bars.

Intra-specific movement of ToxA

The *ToxA* containing chromosome 6 of the reference isolate Pt-1C-BFP fully aligned with the homologous contig 17 of I73-1, with the notable exception of *ToxA*. BLAST results indicated *ToxA* was present on the contig 3 of I73-1. Subsequent alignment and plotting of the three chromosomes revealed a clear translocation event. In-depth analysis of this regions, which spans 143 kbp, revealed short-direct repeats on the edges suggesting that this is a transposon. Additional alignments showed a clear insertion site on Pt-1C-BFP chromosome 1 which is partially homologous to I73-1 contig 3. Functional annotations of the genes in this region revealed the presence of a tyrosine recombinase. A large size, borders of short-direct repeats, and the presence of tyrosine recombinase are strong evidence that this transposon is a crypton.

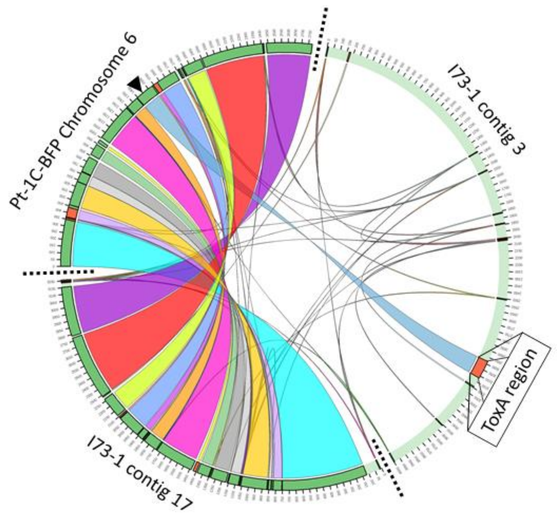

Figure 3. Evidence of the translocation of ToxA containing region between race 1 (Pt-1C-BFP) and race 8 (I73-1) of Pyrenophora tritici-repentis. Size of the 'ToxA region' (red) is ~143 kbp and is bordered by short-direct repeats.

Conclusions

- 40 new high quality short read assemblies
- First long-read assemblies of race 3 (ToxC) and race 8 (ToxA, ToxB, and ToxC)
- Ptr has an 'open' genome and is highly adaptable
  - Large accessory gene count and a large number of gene gains and losses
- Distinct phylogenetic clustering by ability to produce certain efffectors
- Confirmation of ToxA translocation within Ptr
- ToxhAT, a transposon responsible for the horizontal gene transfer of ToxA between different fungal species, is nested within a larger mobile element (likely a crypton)

References

[1]Savary et al., 2019. *Nature Ecology & Evolution*, 3(3), 430; [2]Kamel et al., 2019. *Frontiers in Plant Science,* 10, 1562; [3]Bankevich et al., 2012. *Journal of Computation Biology*, 19(5), 455-477; [4]Seemann, 2019. github.com/tseemann/shovill; [5]Kolmogorov et al., 2019. *Nature Biotechnology* 37(5), 540–546; [6]Walker et al., 2014. *PloS One*, 9(11), e112963; [7]Min et al., 2017. *Bioinformatics* 33(18), 2936-2937; [8]Ou et al., 2019. *Genome Biology* 20(1), 1; [9]McCarthy & Fitzpatrick, 2019. *Genes* 10(7), 521; [10]Stamatakis, 2014. *Bioinformatics* 30(9), 1312; [11]Pedersen 2016. github.com/thomasp85/hierarchicalSets; [12]Krzywinski et al., 2009. *Genome Research* 19(9), 1639