Fungal Genetics Conference

Poster 784A

The replication of the virulence gene *ToxB* in tan spot of wheat

Ryan Gourlie[1,2], Megan McDonald[3], Mohamed Hafez[1], and Reem Aboukhaddour[1]

[1]Agriculture and Agri-Food Canada, LRDC, Lethbridge, AB, Canada; [2]University of Lethbridge, Lethbridge, AB, Canada; [3]University of Birmingham, Birmingham, United Kingdom

Agriculture and Agri-Food Canada

Introduction

The necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Ptr) is a major foliar wheat pathogen with annual global losses estimated at ~5%[1] (Savary et al., 2019). The Ptr-wheat interaction is used as a model system to help understand the evolution of virulence in necrotrophic plant pathogens (those that feed on dead/dying tissues). ToxB is a host-selective toxin (effector), which causes chlorosis symptoms on susceptible wheat. This virulence gene is commonly present as multiple copies within a genome with copy-number being linked to increase symptoms (Strelkov et al., 2002). The multi-copy nature of *ToxB* has remained unexplored in detail since it was first identified in 1999 (Strelkov et al. 1999). The importance of gene duplications in evolution cannot be overstated, with additionally copies being free to diverge, duplications provide genetic material on which selection can act (Lauer et al. 2019). Here we have used long-read assemblies to identify the genomic positions of *ToxB*-copies and found *ToxB* to be present within a semi-conserved region dubbed *Icarus* (Gourlie et al., 2022). Utilizing various types of alignments here we provide evidence that *ToxB* was captured by a Helitron which has subsequently replicated the *ToxB* coding gene, likely in combination with unequal crossover events.

Results and Discussion

Full genome alignments of >20 isolates of *Pyrenophora tritici-repentis* (Ptr) showed that *ToxB* was almost always located near the end of chromosome 4 (based on the reference isolate Pt-1C-BFP) (Figure 1). Comparisons of single-to-muli copy and *ToxB* carriers to non-carriers revealed breaks near the *ToxB* region in some isolates or, in the case of non-carriers, a complete absence of the *ToxB* containing region (Figure 1). This region was ~300 Kb in size but was variable between isolates. Semi-conserved or accessory regions are well known in fungal plant pathogens. These regions often contain virulence factors, such as the Tox1 biosynthetic cluster in *Cochliobolus heterostrophus* (southern corn leaf blight) (Condon et al., 2013). Accessory regions have also been correlated with higher mutation rates, high transposon activity and adaptive evolution. In order to define the replicative unit which contains *ToxB*, self-alignments were utilized to identify the edges (Figure 2). These self-alignments also showed the tandem uni-directional nature of the replication and revealed transposon activity directly adjacent to, and flanking, the *ToxB* replication region (Figure 2). When compared with the single-copy carriers, more transposon activity within the replicative unit was found (Figure 3). This transposon activity included two different Copia-like elements disrupting the *ToxB* open-reading frame, rendering the gene inactive (Figure 3). Examination of the identified edges revealed no long-terminal repeats or terminal inverted repeats common to many transposon classes. Additionally, there was a lack of target-site duplication also present in most types of transposon insertion events. Further analysis revealed hairpin structures on both the 3' and 5' ends of the replicative unit pointing us towards Helitrons. Helitrons lack LTRs, TIRs, and TSDs as we've seen here, they also contain hairpin structures, palindromic sequences and 'TC' dinucleotide on the 5' and and 'CTRR' sequence on the 3' end (Thomas and Pritham, 2015) (Figure 3).

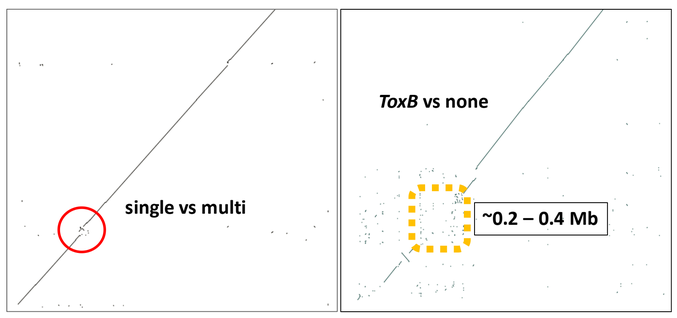

Figure 1. Chromosomes containing *ToxB/toxb* are homolgous to chromosome 4. Isolates with multiple or single copies aligned with isolates containing neither reveal *ToxB* is located within a large semi-conserved region missing from the non-containing isolates.

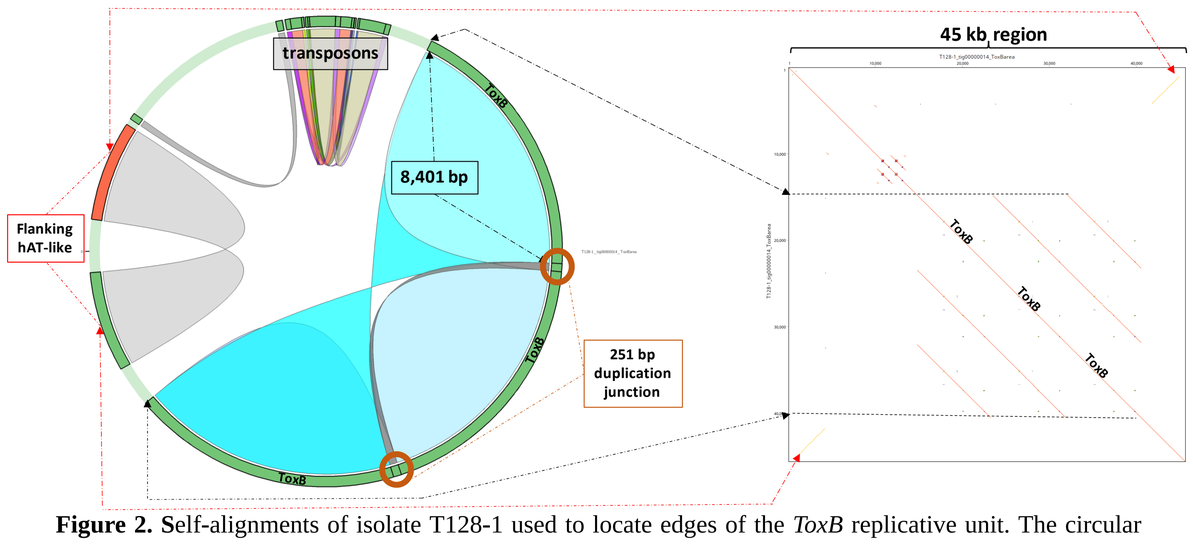

Figure 2. Self-alignments of isolate T128-1 used to locate edges of the *ToxB* replicative unit. The circular alignment shows the tandem replication of the *ToxB* region with duplications separated by a junctions sequence. The linear dotplot shows the unidirectional nature of the duplications and helps locate the specific start and end locations. The alignments also revealed a pocket of nearby transposons and the entire region is flanked by a hAT transposon.

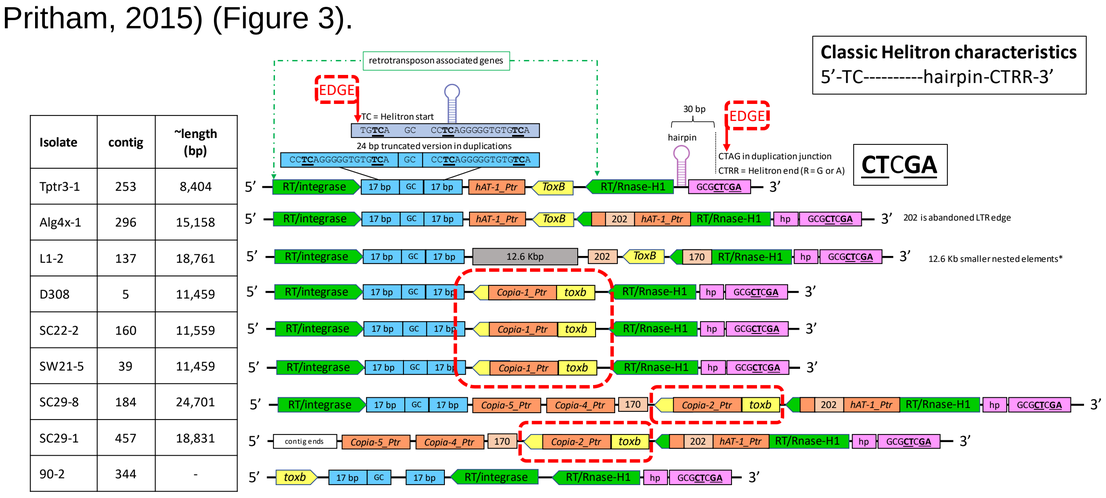

Figure 3. Schematics of region surrounding *ToxB/toxb* in isolates which contain single copies. Features which may help explain *ToxB* replication are noted: yellow = *ToxB/toxb; p*ink = edge of putative replicative unit; blue = other edge; green = retrotransposon related ORF; red = transposon; light red = abandoned LTR edge; hp = hairpin.

Most of the characteristics of Helitrons are present within the *ToxB* replicative unit, with the exception of a slightly altered 3' end. Non-canonical Helitrons with various 3' and 5' ends have been described, specifically in another fungal plant pathogen, *Fusarium oxysporum* (broad host range) (Chellapan et al., 2016). A detailed examination of the multi-copy carriers also reveal the presence of a sequence junction between the replications (Figures 1 and 4). The presence of this sequence junction provides a canonical 3' CTRR end (Figure 4).

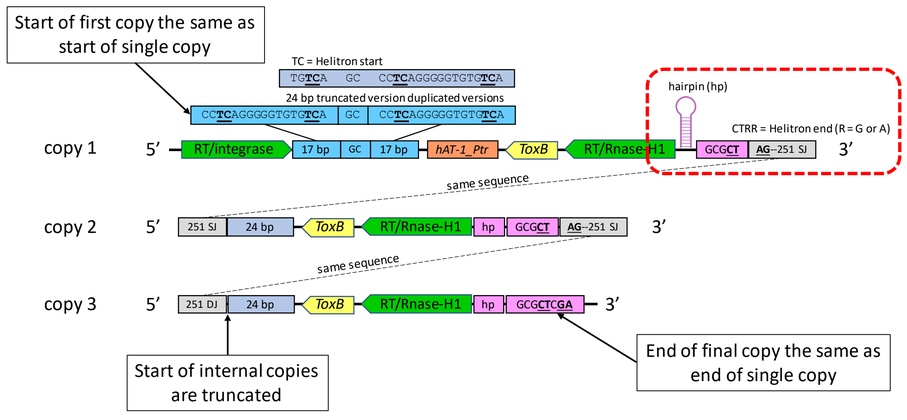

Figure 4. Example schematic of region surrounding *ToxB* in isolates which contain multiple tandem copies. Features which may help explain *ToxB* replication are noted: yellow = *ToxB/toxb; p*ink = edge of putative replicative unit; blue = other edge; green = retrotransposon related ORF; red = transposon; light red = abandoned LTR edge; hp = hairpin.

Conclusion: Our study has provided >20 new long-read genomes of a global plant pathogen. We have provided one of the most comprehensive analyses of virulence gene replication and have provided a framework for understanding mobility and duplication of effectors in a necrotrophic species.

Materials and Methods

Sequencing, assembly, annotation High-molecular weight DNA extracted with 'Genomic-tip 500/G Kit' (Qiagen) and gDNA sequenced with PacBio Sequel I, II, or IIe. Reads assembled with Flye (Kolmogorov et al., 2015), CANU (Koren et al., 2017) or Hi-Canu (Nurk et al., 2020). Gene annotations were done FunGAP (Min et al., 2017) and ORFs identified with Geneious (Kearse et al., 2012).

Alignments Linear alignments between isolates done with Mauve (Darling et al., 2010), Minimap2 (Li, 2018), MAFFT (Katoh and Standley, 2013), and Geneious (Kearse et al., 2012). Circular alignments of *ToxB* carrying chromosomes or the region around *ToxB* done with Sibelia (Minkin et al., 2013) and Circos (Krzywinski et al., 2009).

Transposon annotation Identification of transposon characteristics such as LTR/TIR, CTRR 3' end, hairpins, etc. were performed manually using the various alignments between single-copy, multi-copy, and no-copy isolates.

References

Savary et al., 2019. *Nat Ecol Evol* 3(3):430-439; Strelkov et al., 2002. *Can J Plant Pathol* 24:29–35; Strelkov et al., 1999. *MPMI* 12(8):728-732; Lauer et al., 2019. *Curr Genet* 65(2):1287-1295; Gourlie et al., 2022. *BMC Biol* 20(1):1-21; Condon et al., 2013. PLoS Genet 9(1):e1003233; Thomas and Pritham, 2015. *Mobile DNA* 3: 891-924; Chellapan et al., 2016. *Mobile DNA* 7:1-16; Kolmogorov et al., 2019. *Nat Biotech* 37(5):540–546; Koren et al., 2017. *Genome Res* 27(5):722-736; Nurk et al., 2020. *Genome Res* 30(9):1291-1305; Min et al., 2017. *Bioinf* 33(18):2936–2937; Kearse et al., 2012. Bioinf 28(12):1647-1649; Darling et al., 2010. *PLoS ONE* 5(6):e11147; Li, 2018. Bioinf 34(18):3094-3100; Katoh and Standley, 2013. *Mol Biol Evol* 30(4):772-780; Minkin et al., 2013. *Algorithms Bioinform* Springer; Krzywinski et al., 2009. *Genome Res* 19(9):1639