Genome Assembly and Visualization of Aggressive Wheat Blast Strain 16MoT01

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Research Question

How is the genome of newly emerged wheat blast strain 16MoT01 different from more established, less aggressive strains?

Introduction

- Wheat blast is a destructive disease caused by fungus *Magnaporthe oryzae Triticum* (MoT).
- Recent outbreaks of the fungus caused by a branch of pandemic strains in Asia and Africa threaten the global wheat supply.
- Recently collected strain 16MoT01 overcomes resistance of many wheat genotypes.
- **To begin the process of understanding how this strain undermines wheat resistance, we created a genome assembly of strain 16MoT01 and compared it to the reference genome.**

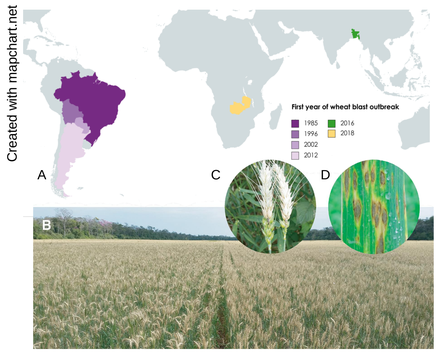

Figure 1. A) countries with wheat blast, labeled by year of emergence – Brazil in 1985, Bangladesh in 2016, and Zambia in 2018.[1] **B)** a Bolivian wheat field, 2015, with near 100% killed heads. **C)** Infected wheat heads. **D)** Sporulating leaf lesions.[2]

Input Data

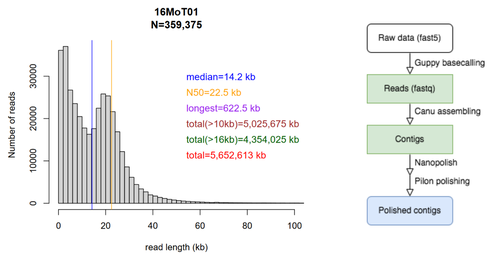

Figure 2: the distribution of read lengths and the genome assembly procedure. The longest reads covering 40x genomes were used for the assembly.

References

1. Singh Pawan K, et al. (2021) Wheat Blast: A Disease Spreading by Intercontinental Jumps and Its Management Strategies. Frontiers in Plant Science: vol 12. https://doi.org /10.3389/fpls.2021.710707
2. Peng Z, et al. (2019) Effector gene reshuffling involves dispensable mini-chromosomes in the wheat blast fungus. PLoS Genet 15(9): e1008272. https://doi.org /10.1371/journal.pgen.1008272

Comparison to Reference Genome

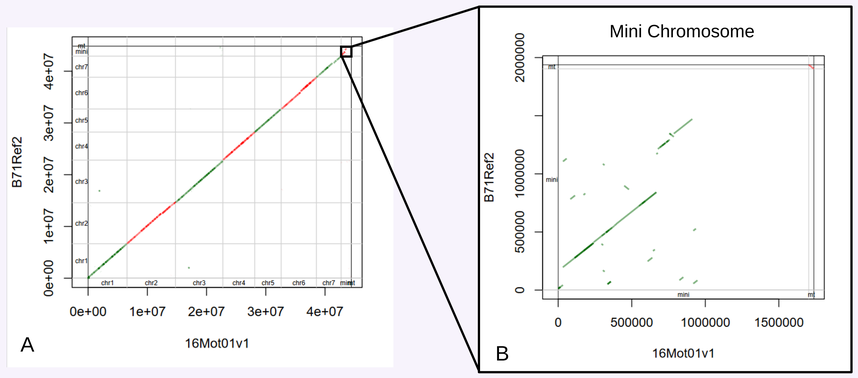

Figure 3: A) The dotplot comparing the 16MoT01 strain to reference genome from strain B71[2] identifies seven core-chromosomes, one mini-chromosome, and one circular mitochondrial chromosome. **B)** The mini-chromosomes show high variability between strains.

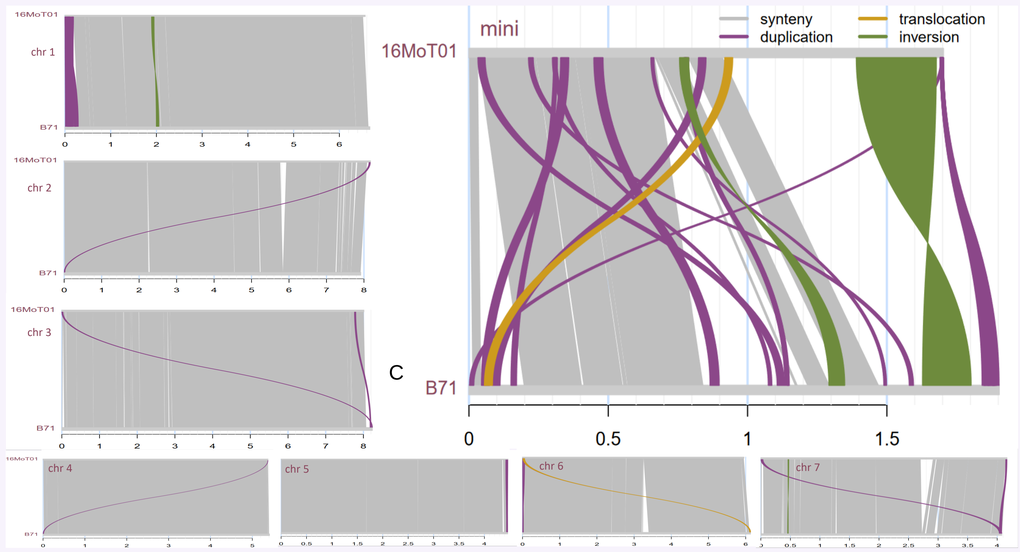

Figure 4. Aligning the genome assembly to the reference genome shows gene movement – the core chromosomes (1-7) are mostly similar (show synteny) and the mini chromosomes have many variations.

Methods

- Wheat blast cultures were stored in Biosecurity Lab;
- DNA was sequenced with Oxford Nanopore sequencing, assembled with Canu at 40x coverage, polished with Illumina reads and pilon, and compared to reference genome B71 with software tools of Nucmer and Syri.

Acknowledgements

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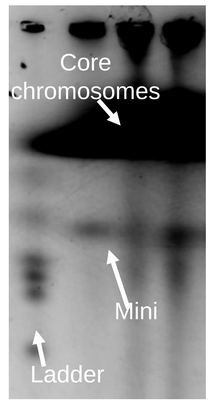
A Look at the Genome



chr	size (bp)
chr 1	6,565,471
chr 2	8,176,520
chr 3	8,013,725
chr 4	5,375,111
chr 5	4,465,290
chr 6	5,955,100
chr 7	4,167,432
mini	1,703,074
mt	34,996

B

Figure 5. A) Rice blast strain O135 run using CHEF gel electrophoresis. The mini chromosomes for this strain are estimated to be about 2.2 Mb. **B)** In 16MoT01, the mini chromosome is 1.7Mb.

The *M. oryzae* genome is divided into core chromosomes and mini-chromosomes, which are thought to be the source of *M. oryzae*'s adaptability. The assembly suggests this strain has one mini-chromosome; this will be verified using contour-clamped homogenous electric field (CHEF) gel electrophoresis.

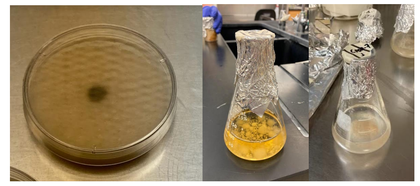


Figure 6. Rice blast being cultured in preparation for CHEF gel electrophoresis, first on oatmeal agar plates and then in liquid media.

Summary

- Wheat blast strain 16MoT01 was sequenced and an assembly was generated, which was then compared to the reference genome
- Seven core-chromosomes, one mini-chromosome, and one circular mitochondrial genome were assembled.
- Core-chromosomes between species were highly similar and mini-chromosomes were highly variable
- CHEF procedure began to be developed for verifying presence of mini-chromosomes

Further Research

- The 16MoT01 genome assembly will be used to compare with additional MoT genomes.
- CHEF protocol for this species will continue to be optimized