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Analysis of small RNA silencing in Zymoseptoria tritici - wheat interactions

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1. Cross-kingdom gene silencing

The ascomycete fungus Zymoseptoria tritici is the causative agent of Septoria tritici blotch (STB) disease and is a major threat to wheat production globally (Dean et al., 2012). Z. tritici is a hemibiotrophic foliar pathogen, which invades leaf tissue through natural openings such as stomata. Z. tritici remains exclusively apoplastic through its infection cycle, which is characterized by a long symptomless infection phase (10-14 days), followed by the transition to necrotrophy (Kema et al., 1996). Recent observations suggest that natural cross-kingdom gene silencing (RNAi) can occur in some plant-fungal interactions (Wekberg et al., 2013; Yang et al., 2016). These developments prompted us to ask whether this phenomenon was involved in the colonization of wheat by Z. tritici, an exclusively apoplastically-dwelling pathogen. The existence of fungal sRNA in Z. tritici is to date unexplored. Here we report our findings to characterize the sRNA populations of this fungal species during wheat leaf infection, predict putative wheat transcripts that may be subject to cross-kingdom RNAi and biochemically validate such interactions.

2. sRNA discovery in Z. tritici

The genome of Z. tritici isolate IPO323 is predicted to encode a single Dicer (ZtDCL - Mycgr3G47982) and two Argonautes (ZtAGO1 - Mycgr3G8035, ZtAGO2 - Mycgr3G6021) ribonucleases. This provides indirect evidence that a system for sRNA biogenesis may exist in Z. tritici. We therefore sequenced sRNA preparations from in vitro cultured fungus, and from infected susceptible wheat plants (cv. Bobwhite) over a 21-day timecourse (Fig.1).

3. Transcriptional profiling of Z. tritici sRNAs and putative wheat targets

We identified 262 fungal sRNAs computationally predicted to target 737 wheat transcripts. It was expected that wheat transcripts successfully targeted by fungal sRNAs would display downregulation during some or all stages of infection. We prioritized ten wheat mRNAs (Table 1), predicted to be targeted by four Z. tritici sRNAs (by mRNA cleavage) for investigation based on expression profile (Fig.2) and role in pathogen defense.

4. Z. tritici RNAi mutants are fully pathogenic

To examine the role of the fungal RNAi pathway in the interaction between wheat and Z. tritici, we produced several fungal mutants deficient in key RNAi components (Fig.4). These experiments demonstrated that the ZtDCL, ZtAGO1, and ZtAGO2 genes are dispensable for virulence.

5. Dicer-independent sRNA production in Z. tritici

We carried out wheat infection time courses with Z. tritici IPO323, blu70 (control) and the ∆dcl mutant followed by fungal sRNA expression analysis (Fig.5). Expression of some fungal sRNAs was maintained in the ∆dcl mutant indicating DCL-independent sRNA biogenesis in Z. tritici (Fig.5). Expression analysis of a wheat mRNA target for the DCL-dependent ZtRNA2 showed no difference between, blu70 (control) and ∆dcl mutant infections (Fig.6), indicating that this mRNA is unlikely to be a genuine sRNA target.

6. Host-induced gene silencing (HIGS) and in vitro dsRNA uptake are ineffective against Z. tritici

To test whether plant-derived sRNAs could translocate and induce RNAi in Z. tritici during infection, we used a plant RNA virus-based vector as an RNA silencing inducer in a procedure known as HIGS (Fig.7). In parallel, we investigated whether external application of dsRNAs targeting essential for life fungal genes could restrict Z. tritici growth in vitro (Fig.8). To directly assess the uptake of dsRNA by Z. tritici, we co-incubated germinating conidiospores with fluorescently-labelled short or long dsRNA.

Conclusions

- Z. tritici encodes sRNAs that are transcriptionally-induced during infection but we could not validate targeting of wheat mRNAs
- RNAi pathway is dispensable for fungal virulence
- Some Z. tritici sRNAs are generated by an unknown Dicer-independent mechanism
- Unlikely Z. tritici can absorb functional sRNA from host plants or environment
- RNAi likely to be less effective at control of Z. tritici than for some other fungal pathogens

References


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