

## Abstract

The mechanisms underlying plant–microbe interactions are typically investigated using bilateral models. This approach has provided important insights into plant immunity and microbial infection strategies, but it is now clear that such interactions are much more complex in nature and are largely regulated by the plant microbiome. Therefore, we hypothesized that microbe–microbe competitions are key processes affecting plant–microbe interactions and are possibly driven by microbial effector functions. The basidiomycete *Serendipita vermifera* (MAFF 305830) confers a broad spectrum of beneficial effects on its hosts, but it can also complete part of its life cycle outside the host saprotrophically suggesting that it has probably evolved strategies to combat microbial competitors in the rhizosphere. On the other hand, the ascomycete *Bipolaris sorokiniana* is a cereal pathogen that causes severe infections in monocotyledonous plants. The importance of root-inhabiting phytopathogenic fungi is often underestimated, and although *B. sorokiniana* infects roots almost as often as shoots, comparatively little is known about its interactions with the roots of host plants. Based on the ability of *S. vermifera* to antagonize the pathogen *B. sorokiniana* in direct confrontation assays, we focused on studying the bioprotective activity of *S. vermifera* in barley roots infected with *B. sorokiniana*. We established a gnotobiotic split root system based on natural soil, which allowed us to investigate the local and systemic aspects of the multispecies interaction. Analysis at the phenotypic, cytologic and transcriptomic levels revealed that the presence of *S. vermifera* locally in the infected root reduced the biomass of *B. sorokiniana* and consequently the severity of disease symptoms in barley roots. In order to determine the host response to fungal invasions, we found that barley responds differently to the pathogen and the symbiont. Furthermore, co-invasion activated host stress tolerance as well as defence associated regulatory genes including heat stress transcription factors (HSFs) and the zinc-finger protein CONSTANS-LIKE 9. On the other hand, presence of the symbiont systemically altered the localized host response (particularly Fe homeostasis) to *B. sorokiniana*, although this had no statistically significant effect on pathogen biomass. In the context of fungus-fungus interaction, *S. vermifera* potentially can antagonise *B. sorokiniana* without the host plant. Direct hyphal contact resulted in the upregulation of the secondary metabolism in *B. sorokiniana*, whereas most of the genes modulated in *S. vermifera* encoded proteins with hydrolytic activities, highlighting the different competitive strategies of the two

fungi. Although antagonism-related genes (including a chitinase) in *S. vermifera* were upregulated during direct confrontation with *B. sorokiniana* in soil, the same genes were not induced during confrontation *in planta*, indicating that different antagonistic strategies are used in the two environments. However, some potential antagonism-associated *S. vermifera* genes were activated in both situations such as those encoding glutathione-S-transferase, heat shock protein (HSP) 9/12, cytochrome P450, LysM domain protein, glucose oxidase and proteins with carbohydrate binding module (CBM) 13 domain indicating the possible role of fungal antagonism underlying the bioprotective effect. These findings provide the basis for the characterization of multispecies interactions in terms of the genes expressed by each participant, and shed light on the mechanisms involved in fungal antagonism inside and outside the roots.