

Comparative chemical screening and genetic analysis reveal tentoxin as a new virulence factor in *Cochliobolus miyabeanus*, the causal agent of brown spot disease on rice

Abstract

Brown spot disease, caused by *Cochliobolus miyabeanus*, is currently considered to be one of the most important yield reducers of rice (*Oryza sativa* L.). Despite its agricultural importance, little is known about the virulence mechanisms deployed by the fungus. Therefore, we set out to identify novel virulence factors with a role in disease development. This article reports, for the first time, the production of tentoxin by *C. miyabeanus* as a virulence factor during brown spot disease and the identification of the non-ribosomal protein synthetase (NRPS) CmNps3, responsible for tentoxin biosynthesis. We compared the chemical compounds produced by *C. miyabeanus* strains differing in virulence ability using ultra-high-performance liquid chromatography (UHPLC) coupled to high-resolution Orbitrap mass spectrometry (HRMS). The production of tentoxin by a highly virulent strain was revealed by principal component analysis of the detected ions and confirmed by UHPLC coupled to tandem-quadrupole mass spectrometry (MS/MS). The corresponding NRPS was identified by *in silico* genome analysis and confirmed by gene deletion. Infection tests with wild-type and *Cmnps3* mutants showed that tentoxin acts as a virulence factor and is correlated with chlorosis development during the second phase of infection. Although rice has previously been classified as a tentoxin-insensitive plant species, our data demonstrate that tentoxin production by *C. miyabeanus* affects symptom development.

Keyword: Bipolaris oryzae; Cochliobolus miyabeanus; Oryza sativa L.; Brown spot disease; Plant-pathogen interactions; Rice; Tentoxin