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Functional analysis of cassiicolin, effector of the rubber tree pathogen *Corynespora cassiicola*

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Rubber tree (*Hevea brasiliensis*) is the main source of natural rubber around the world. In Africa and Asia, it is affected by the *Corynespora* Leaf Fall disease caused by the necrotrophic fungus *Corynespora cassiicola*. Some strains of this pathogen secrete a small protein toxin, the cassiicolin, which play a role in the early stages of disease establishment.

In this study, we demonstrated the importance of cassiicolin for aggressiveness, by comparing the wild-type virulent CCP strain and the same strain deleted for the cassiicolin-encoding gene *Cas1* (CCPΔ*Cas1*), in interaction with rubber tree.

Deletion of *Cas1* gene did not modify major functions in the CCP strain: the growth rate, conidia production and percentage of germination were found similar for both strains. The wild-type and mutated strains were compared for their virulence on different rubber clones by analyzing the extent of symptoms induced after application of conidia suspension. Only hardly detectable lesions were observed with CCPΔ*Cas1*, suggesting that cassiicolin *Cas1* is an important factor of virulence in rubber tree. Moreover, the two strains were compared for filtrate toxicity over a larger range of rubber clones, by measuring the electrolyte leakages induced on detached leaves by the application of purified cassiicolin *Cas1* or culture filtrate from several *C. cassiicola* strains (including CCP and CCPΔ*Cas1*). The filtrate of the mutated strain was on average less aggressive than the wild-type filtrate although it still generated severe symptoms on a few clones, suggesting the involvement of toxicity factors other than cassiicolin. Hierarchical clustering (clones x treatments) based on these toxicity profiles grouped CCPΔ*Cas1* with strains of a different phylogenetic group (clade A) compared to CCP (clade C), without cassiicolin-encoding gene. These results demonstrate that, despite the production of other effectors by CCP, cassiicolin *Cas1* is a major determinant of this strain controlling the virulence level in rubber tree.

Keywords: *Corynespora cassiicola*, rubber tree, cassiicolin, effector, deletion mutant