Deletion of the cassiicolin-encoding gene Cas1 from Corynespora cassiicola causes a loss of virulence on rubber tree

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Background

Rubber tree (Hevea brasiliensis) is an economically important tropical tree that provides natural rubber. In Asia and Africa, it is affected by the Corynespora Leaf Fall (CLF) disease caused by the necrotrophic fungus Corynespora cassiicola. On the most susceptible rubber clones, C. cassiicola can cause massive defoliation leading to yield losses. Some pathogenic strains secrete a small protein toxin, the cassiicolin\textsuperscript{1-3}, which is transcriptionally up-regulated during early stages of the disease\textsuperscript{4}. Seven isoforms were identified and used to classify the various strains into toxin classes\textsuperscript{5}. Strains belonging to toxin class Cas1 were the most aggressive on tested rubber clones\textsuperscript{1}. However, strains without cassiicolin gene (toxin class Cas0) may also produce toxic exudates\textsuperscript{6}, suggesting the existence of effectors other than cassiicolin.

Objective

To determine the importance of cassiicolin Cas1 for the virulence of C. cassiicola strain CCP, comparatively to others putative effectors, by comparing the wild-type strain and the same strain deleted for the cassiicolin-encoding gene Cas1, in interaction with rubber tree.

Construction of the Cas1 gene deletion mutant

Deletion mutant was obtained by replacing the Cas1 gene from the highly aggressive strain CCP with a hygromycin-resistance cassette by homologous recombination. The deletion mutant was named ccpΔcas1.

Virulence of the ccpΔcas1 strain

CCP and ccpΔcas1 strains were compared for their virulence on four rubber clones (still attached to the plant), by analyzing the extent of symptoms as the mean surface of necrotic tissue (mm\textsuperscript{2}), nine days after inoculation (200 conidia/drop, 6 drops per leaf).

QTL associated with sensitivity of rubber tree to cassiicolin Cas1

QTL detection was conducted on the PB260 x RRIM600 family for the response to purified cassicinoline Cas1, to culture of isolates carrying or not a Cas1 gene (including CCP and ccpΔcas1) and to blank treatments (culture medium and water). The response was quantified by conductivity measurement of the induced electrolyte leakage\textsuperscript{6}.

Physiological analyses of CCP and ccpΔcas1

The growth rate, conidia production and percentage of germination were compared in vitro between CCP and ccpΔcas1 strains.

Result 1: Deletion of the Cas1 gene did not modify major physiological functions in the CCP strain.

Result 2: Without cassiicolin Cas1, the CCP strain is avirulent on susceptible clones, except for a few pinpoint symptoms.

Result 3: At least two QTL (in yellow) were detected with the purified cassicinoline and with Cas1 culture filtrates, but none with ccpΔcas1.

Conclusion

Cassicinoline Cas1 is the major virulence factor involved in the compatible interaction between CCP and susceptible rubber clones. It could be a good candidate for effector-based selection.

References