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

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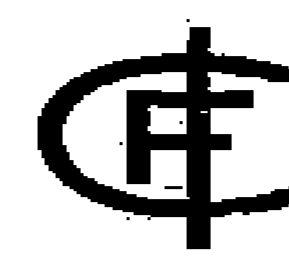
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Background

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 yield losses. Some pathogenic strains secrete a small protein toxin, the **cassiicolin**^{1,2,3}, which is transcriptionally up-regulated during early stages of the disease⁴. Six isoforms were identified and used to classify the various strains into **toxin classes**⁵. However, *C. cassiicola* strains without cassiicolin gene (toxin class Cas0) may also produce toxic exudates⁶, suggesting the existence of effectors other than cassiicolin.



susceptible clone



tolerant clone

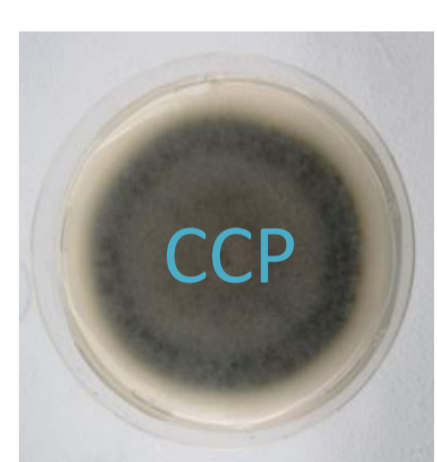
Objective

To determine the **importance of cassiicolin Cas1 for virulence of *C. cassiicola***,

by comparing the wild-type virulent CCP strain and the same strain deleted for the cassiicolin-encoding gene *Cas1*, in interaction with rubber tree.

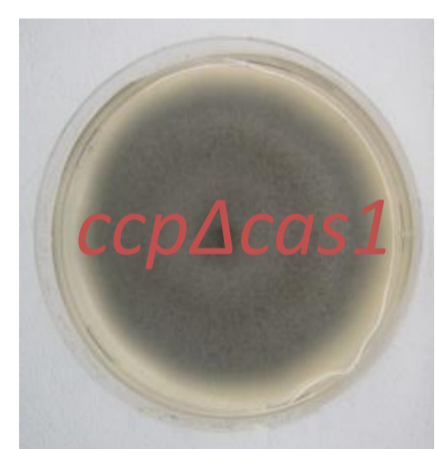
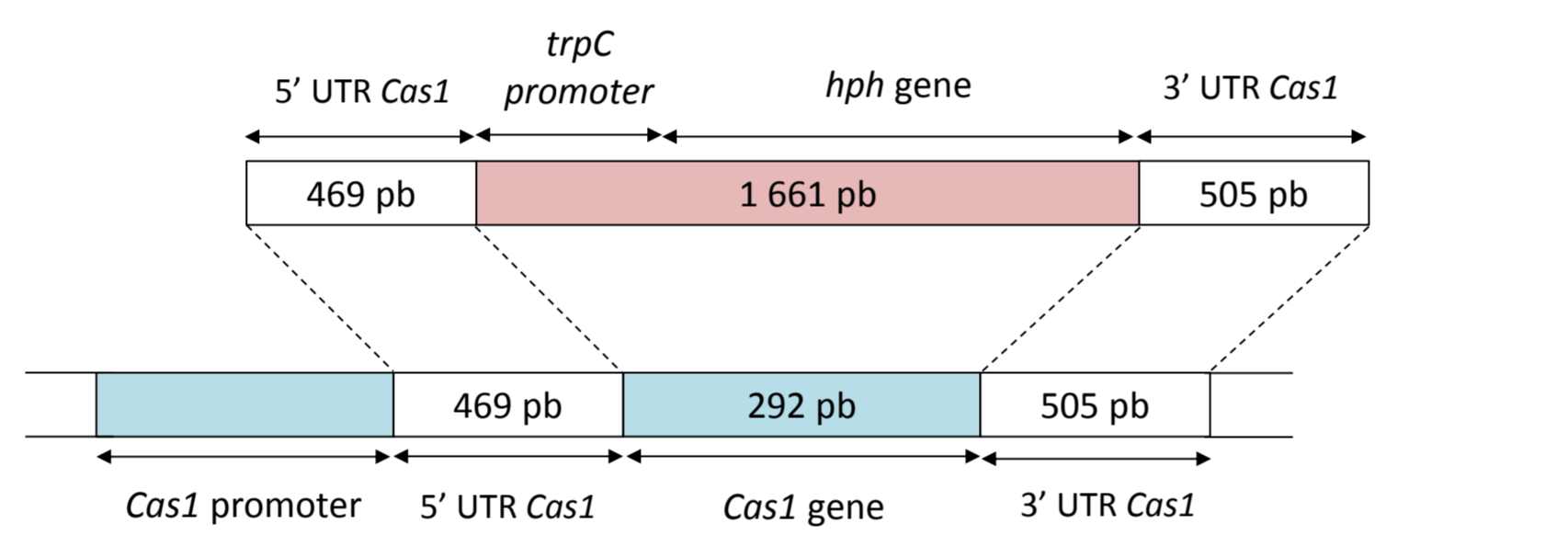
Construction a *Cas1* gene deletion mutant

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

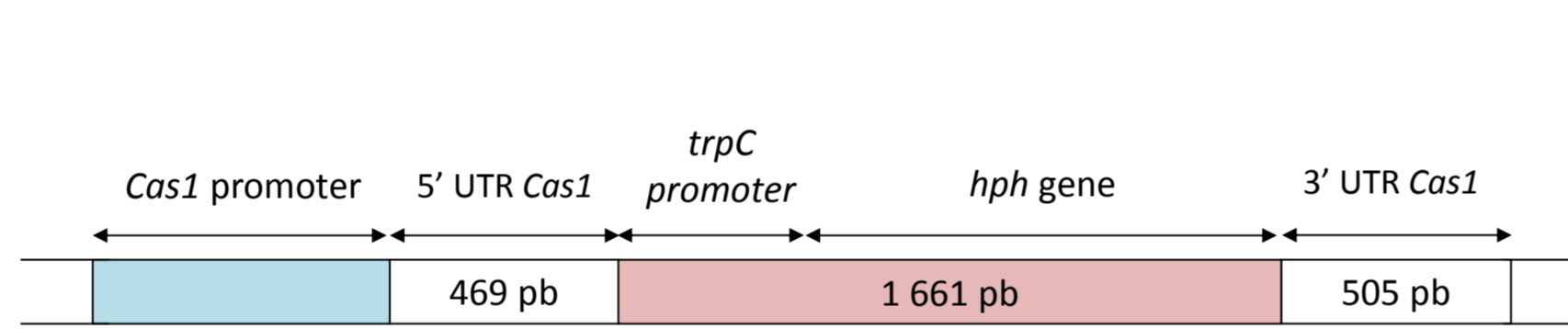


hygromycin cassette

wild-type
CCP genome



deletion mutant
ccpΔcas1 genome



Comparison between CCP and *ccpΔcas1*

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

Strain	Growth rate (mm/day)	Conidiation (conidia/μl)	Germination (%)
CCP	4.13 ± 0.16a	413 ± 77a	71.75 ± 9a
<i>ccpΔcas1</i>	4.29 ± 0.08a	479.5 ± 42a	69.75 ± 6a

→ Deletion of the *Cas1* gene did not modify major physiological functions in the CCP strain.

Toxicity of *ccpΔcas1* filtrate

Eighteen rubber clones were tested for their sensitivity to *C. cassiicola* exudates, by conductivity measurement of the induced electrolyte leakage (%EL)⁶.

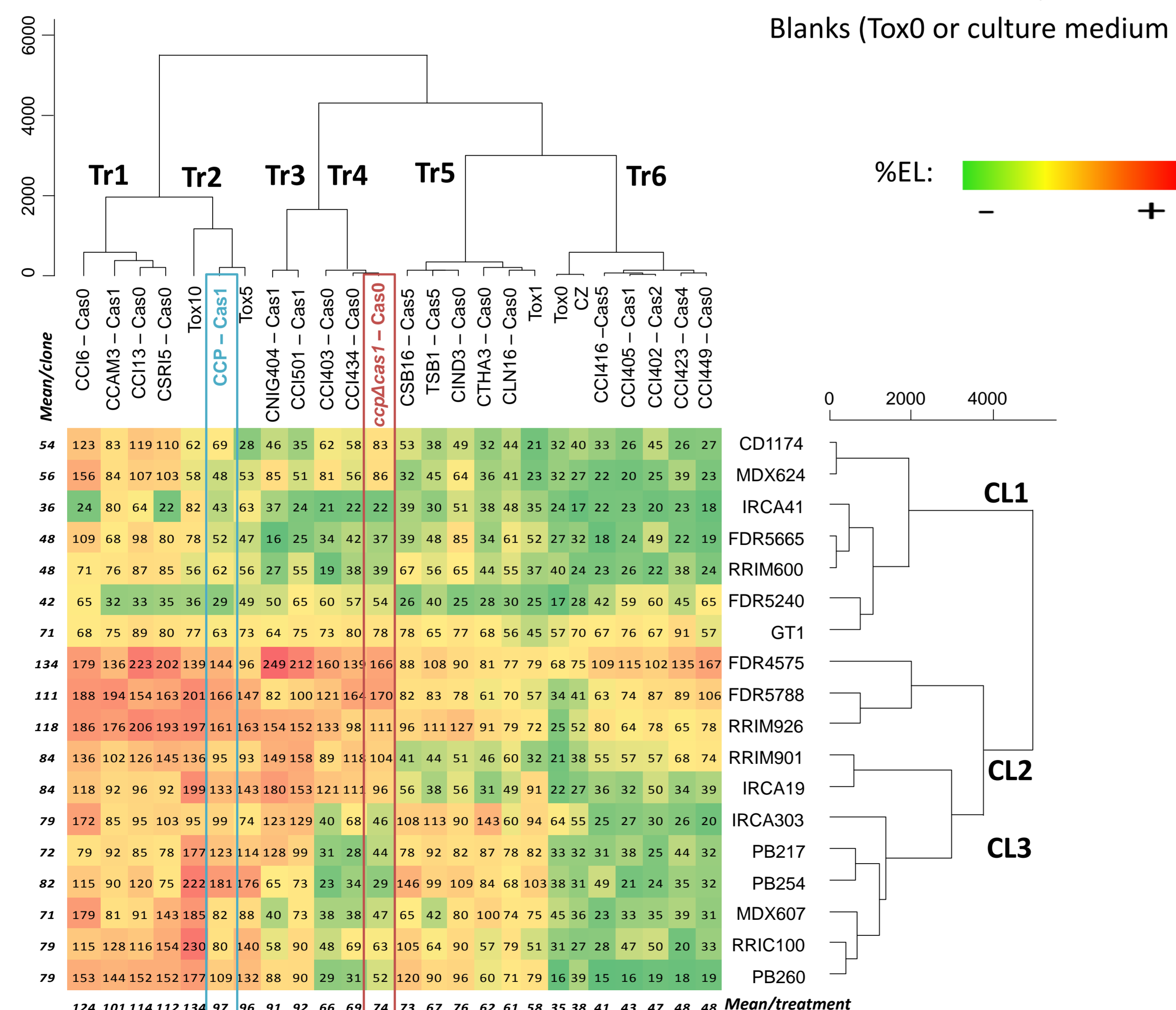
Hierarchical clustering = Clones (CL) x Treatments (Tr)

Treatments:

Culture filtrates (strain name – toxin class)

Purified cassiicolin at various concentration (Tox1, 5 or 10)

Blanks (Tox0 or culture medium Cz)



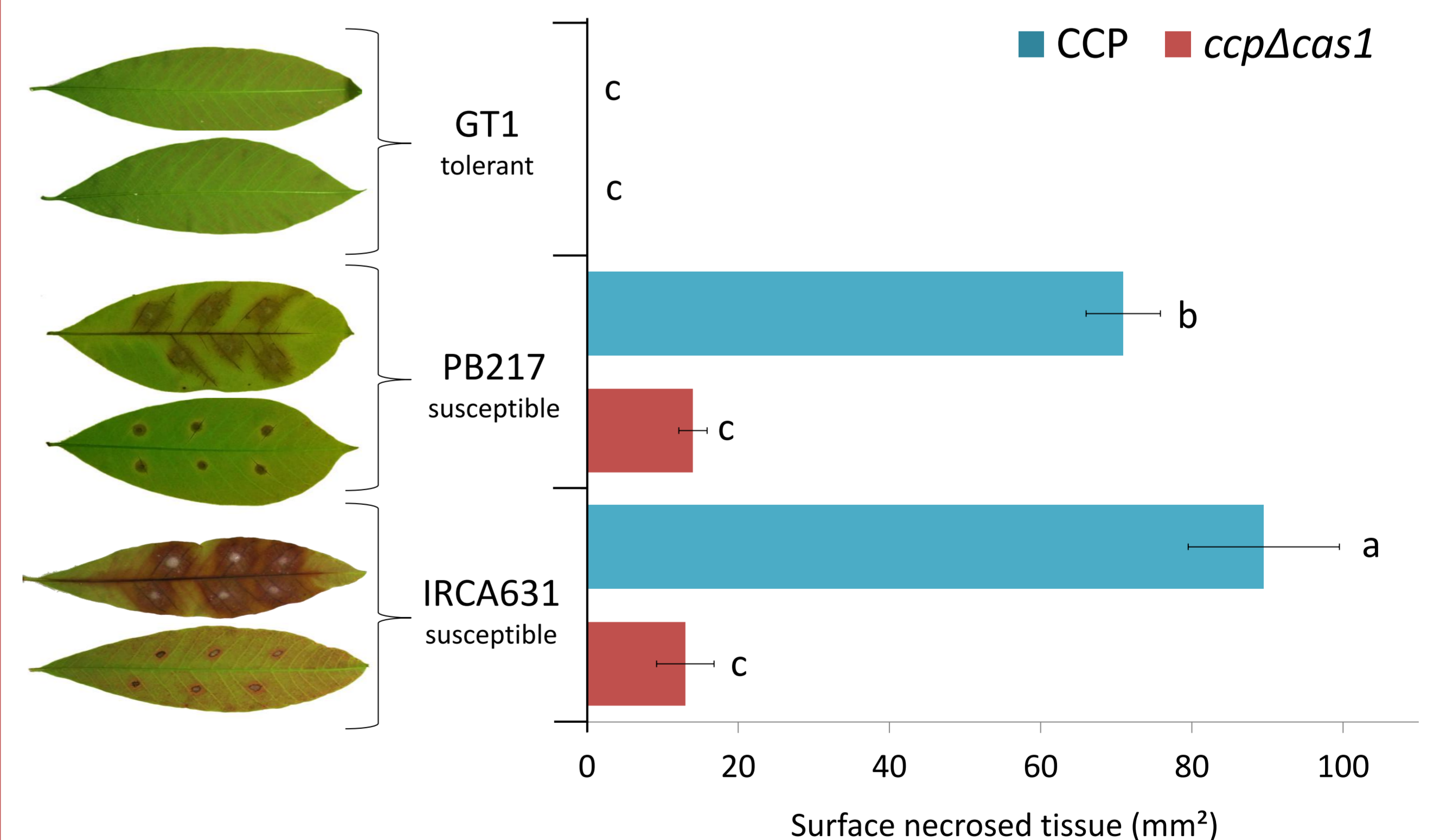
→ Both CCP and *ccpΔcas1* filtrates are toxic, although with different profiles:

CCP is clustered in Tr2, with Tox5 and Tox10

ccpΔcas1 is clustered in Tr4, with Cas0 strains

Pathogenicity of *ccpΔcas1* strain

CCP and *ccpΔcas1* strains were compared for their virulence on detached leaves of three rubber clones, 4 days after application of conidia (50 conidia/μl, 6 drops per leaves):



→ Without cassiicolin *Cas1*, the CCP strain is almost avirulent and doesn't extend beyond the inoculation spot.

Conclusion

Cassiicolin *Cas1* is the major virulence factor involved in the compatible interaction between CCP and rubber clones, despite the presence of other putative effectors causing filtrate toxicity.

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