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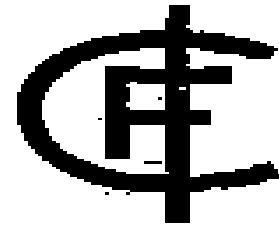
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# Deletion of the cassiicolin-encoding gene *Cas1* from *Corynespora cassiicola* causes a loss of virulence on rubber tree

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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**<sup>1,2,3</sup>, which is transcriptionally up-regulated during early stages of the disease<sup>4</sup>. Seven isoforms were identified and used to classify the various strains into **toxin classes**<sup>5</sup>. Strains belonging to toxin class **Cas1** were the most aggressive on tested rubber clones<sup>5</sup>. However, strains without cassiicolin gene (toxin class **Cas0**) may also produce toxic exudates<sup>6</sup>, suggesting the existence of effectors other than cassiicolin.



susceptible clone      tolerant clone

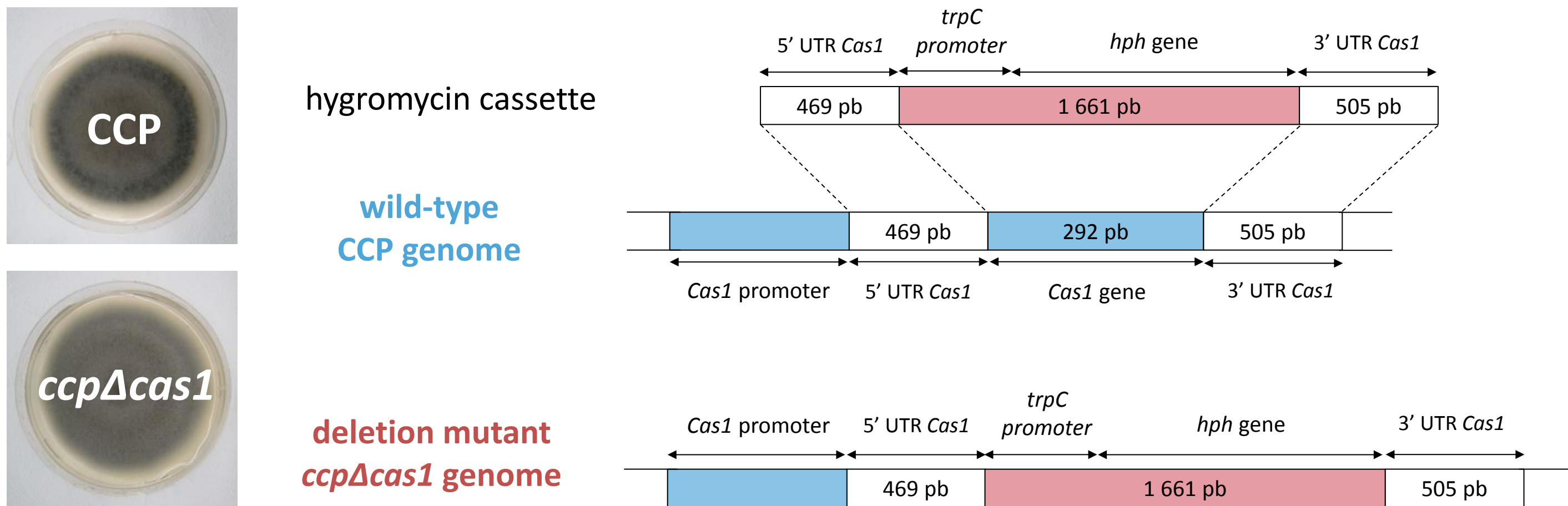
## Objective

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***.



## Physiological analyses of CCP and *ccpΔcas1*

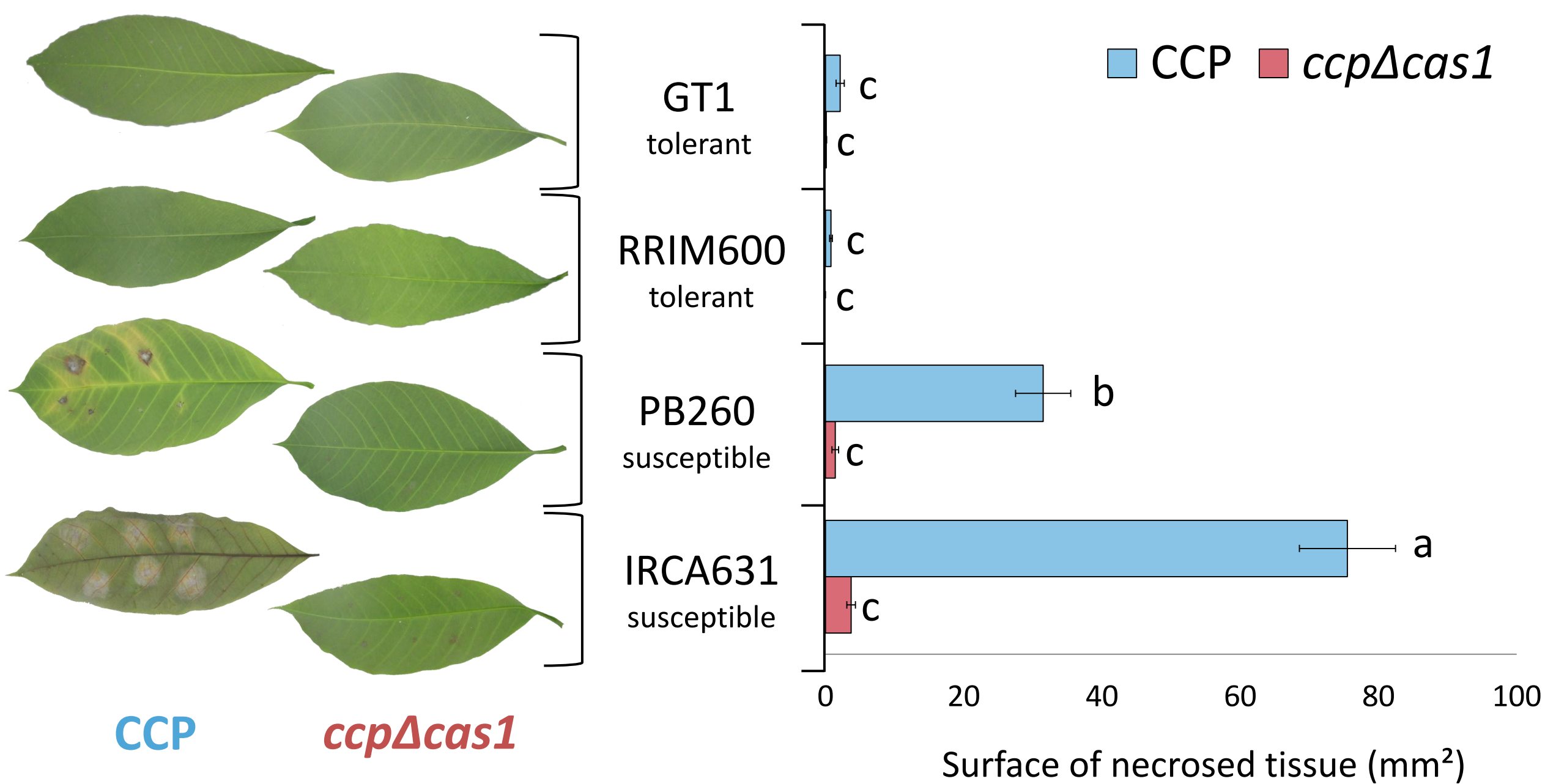
The growth rate, conidia production and percentage of germination were compared *in vitro* between **CCP** and ***ccpΔcas1*** strains.

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

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

## 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<sup>2</sup>), nine days after inoculation (200 conidia/drop, 6 drops per leaf).



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

## 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 cassiicolin 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<sup>6</sup>.

Treatment	g2-26	g3-11	g4-95	g4-32	g5-73	g6-26	g9-62	g9-122	g12-53	g13-102	g14-64	g16-11	g16-77	g18-87
Purified cassiicolin Cas1	<b>17</b>	-	<b>17</b>	-	-	-	-	-	-	-	-	-	-	-
<b>CCP - Cas1</b>	<b>12</b>	-	<b>13</b>	-	<b>14</b>	-	-	-	-	-	-	-	-	-
<b><i>ccpΔcas1</i> - Cas0</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCi501 - Cas1	<b>16</b>	-	<b>19</b>	-	-	-	-	-	-	-	-	-	-	-
CNig404 - Cas1	<b>20</b>	-	<b>12</b>	-	-	-	-	-	-	-	-	-	-	-
CCi434 - Cas0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CCi403 - Cas0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIND3 - Cas0	-	-	-	-	-	-	<b>11</b>	-	-	-	-	<b>11</b>	-	-
CLN16 - Cas0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CTHA3 - Cas0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Culture medium (blank)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Water (blank)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Toxin class (Cas1 or Cas0) is indicated for each isolate.  
QTL are named by the number of the linkage group (g) on which they are located, and their position.  
Numbers represent the percentage of explained phenotypic variance.

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

## Conclusion

Cassiicolin 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**.

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