













Chitinase Dynamics and Resistance Mechanisms in **Coffee Leaf Rust Infection**

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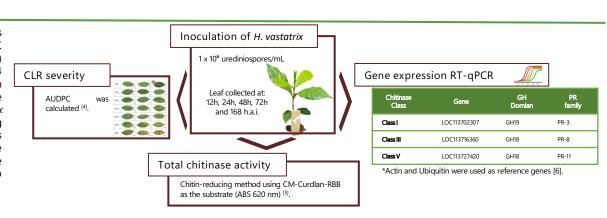
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Introduction

In plants, pathogen recognition through PTI and ETI triggers defense responses, including the production of chitinases—antifungal enzymes that degrade fungal cell walls. Chitinases^[1], classified into GH18 and GH19 families and five structural classes (I–V), play key roles in pathogen resistance^[2,3]. This study examines chitinase activity and gene expression in two Coffea arabica cultivars, Arara (resistant) and Catuaí IAC 144 (susceptible), to investigate their responses to coffee leaf rust (CLR).

Materials/Methods

Two independent experiments were conducted using arabica cultivars Arara (resistant) and Catuaí Vermelho IAC 144 (susceptible) arranged randomized blocks. Plants were inoculated with H. vastatrix urediniospores, and leaves were collected to assess total chitinase activity and gene expression (RT-qPCR). Coffee Leaf Rust severity was also performed.



Results/Discussion

CLR severity

area under the disease progression curve (AUDPC) for the Catuai IAC-144 cultivar inoculated H. vastatrix was 27.29%. indicating severe chlorosis and abundant urediniospore pustules (Fig. 1). In contrast, the resistant cultivar Arara showed no disease symptoms (AUDPC = 0), exhibiting only flecks characteristic of a hypersensitive response (HR) to H. vastatrix.

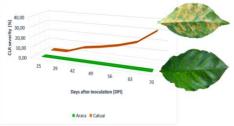


Fig. 1: Coffee Leaf Rust Severity evaluated in C. arabica Arara and Catuaí IAC 144 cultivars.

The clear manifestation of disease in Catuai IAC-144 and its absence in Arara confirmed the validity of the experimental infection, ensuring reliable comparisons of biochemical and molecular defense responses.

Total chitinase activity and gen expression

Total chitinase activity was similar in both cultivars when normalized to the control (Fig. 2). At 48 hours post-inoculation, the susceptible Catuaí IAC 144 showed a significantly greater increase than the resistant cultivar.

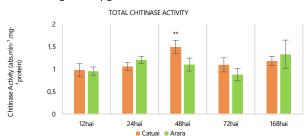


Fig. 2: Temporal profile of total chitinase activity in Arara (resistant) and Catuaí (susceptible) vars inoculated with *H. vastatrix.* Values were normalized by control. Means values were significantly different according to Tukey (p< 0.01 ~ "**").

Gene expression RT-qPCR

Gene expression analysis of chitinase genes revealed clear differences between cultivars (Fig. 3). Chi class I showed higher expression in the susceptible Catual IAC 144, indicating an early response (12-14 h), also observed for Chi class III (24 h). In contrast, Arara showed upregulation of all Chi genes at 48 h, with Chi III and Chi V remaining elevated thereafter. Sustained expression of Chi I, Chi III, and Chi V in Arara suggests an effective antifungal response combining basal and pathogen-induced defenses, whereas in Catual IAC 144 only basal defenses were activated, leading to a less effective response.

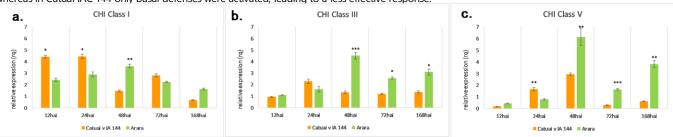


Fig. 3: Expression of chitinase genes (Chi I, Chi III, and Chi V) in Arara (resistant) and Catuaí (susceptible) coffee cultivars.. Values were normalized by control. Means values were significantly different according to Tukey (p< 0.001 ~ "***", p< 0.01 ~ "***", p< 0.01 ~ "**", p< 0.05 ~ "*").

Conclusion/Perspectives

Arara's resistance to coffee leaf rust (CLR) involves sustained, isoform-specific upregulation of Chi III and Chi V, while the susceptible Catuaí IAC 144 shows only transient or early induction of Chi I and Chi III, highlighting the importance of the timing and duration of gene expression. These findings could be applied in the future to breeding programs for CLR-resistant coffee varieties, potentially using marker-assisted selection.

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