Chitosan- and copper-functionalized silica nanoparticles enhance defense-related gene expression in *Coffea arabica* var. Bourbon

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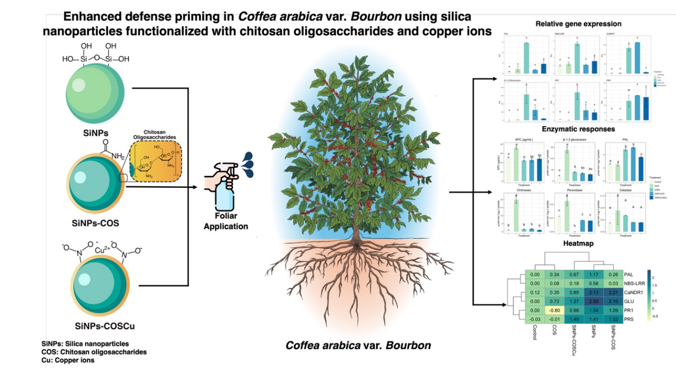
Abstract



Coffee (*Coffea arabica*) is highly vulnerable to biotic stressors that compromise plant health and productivity. This study evaluated the use of silica nanoparticles (SiNPs) functionalized with chitosan oligosaccharides (COS) and copper ions (Cu^{2+}) as foliar elicitors of molecular and biochemical defense responses in *C. arabica* var. *Bourbon*. SiNPs were synthesized via the sol–gel method and characterized by FTIR, DLS, and SEM-EDX. Treatments were applied by foliar spray, and samples were analyzed 72 hours later. The formulations modulated the expression of key defense-related genes (*PAL, PR1, PR5, CaNDR1, NBS-LRR*, and *β-1,3-glucanase*), with *β-1,3-glucanase* increasing up to 314-fold in SiNPs-treated plants and *CaNDR1* up to 163-fold in response to SiNPs-COS. SiNPs-COSCu induced strong expression of *PR1* and *PR5* (34- and 31-fold, respectively). Enzymatic responses showed treatment-specific patterns. PAL activity was highest in SiNPs (4.80 ± 0.23) and SiNPs-COS (5.10 ± 0.08 μmol min⁻¹ μg⁻¹ protein). COS-treated plants exhibited elevated β-1,3-glucanase (137.94 ± 13.36), chitinase (19.90 ± 2.17), and peroxidase (0.02 ± 0.00) activity. Catalase activity was notably elevated in SiNPs-COSCu-treated plants (0.03 ± 0.00), suggesting a ROS-regulating effect linked to copper ions. Physiological responses confirmed membrane protection under SiNPs (EL: 17.99 ± 2.39%; MS: 82.01 ± 2.39%) and SiNPs-COS (EL: 12.95 ± 0.06%; MS: 87.05 ± 0.06%) treatments, while SiNPs-COSCu showed higher EL and reduced MS, indicating oxidative imbalance. These findings highlight the potential of functionalized SiNPs as preventive nanoelicitors capable of enhancing early defense responses in coffee under non-infectious conditions.

Keywords. Plant immunity, Nanobiotechnology, Systemic acquired resistance, Foliar elicitor, Sol-Gel

Introduction

Coffee crops face increasing challenges due to both abiotic and biotic stressors. Among the most critical threats are climate change, nutrient deficiencies, pests, and phytopathogens (García-Méndez et al., 2024).. For instance, elevated temperatures have been associated with increased aggressiveness of the coffee berry borer (*Hypothenemus hampei*) and coffee leaf rust (CLR), caused by *Hemileia vastatrix* (Ayalew et al., 2024; Paragon Ritonga and Kwon, 2024).

Functionalization of SiNPs with biopolymers or metal ions such as Cu^{2+} has been proposed as a strategy to improve the stability, bioavailability, and functional properties of these nanomaterials. Such biofunctionalized nanoparticles can enhance plant uptake, prolong the release of active molecules, and selectively modulate key components of the plant immune system (Sarkar et al., 2022).

This study aims to evaluate the physicochemical properties and biological effects of SiNPs functionalized with COS and Cu^{2+} (SiNPs-COS and SiNPs-COSCu) in *Coffea arabica* var. *Bourbon*. Specifically, we assess their impact on the expression of defense-related genes, enzymatic responses, and cell membrane stability as indicators of immune activation. Our findings provide insights into the design of targeted nanomaterials for sustainable disease management in perennial crops.

Methodology

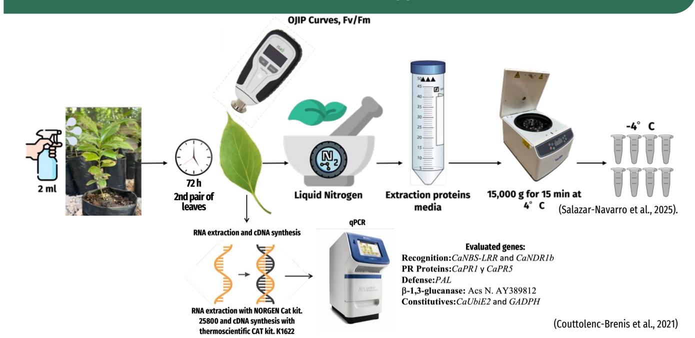


Figure 1. Analysis of biochemical and molecular parameters in *Coffea arabica* Var. Bourbon elicited with SiNPs

Results

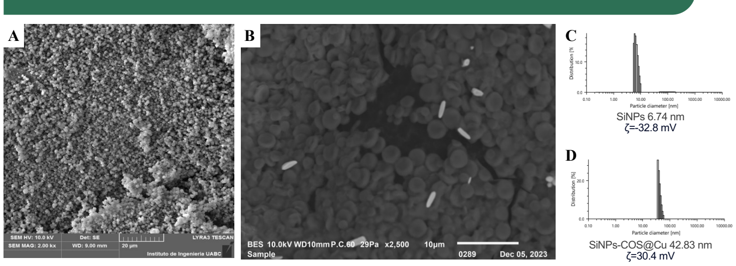


Figure 2. SEM images, EDX and DLS analysis of A) SiNPs and B) SiNPs-COSCu at different magnifications. C) shows DLS parameters of SiNPs and D) SiNPs-COSCu.

Results

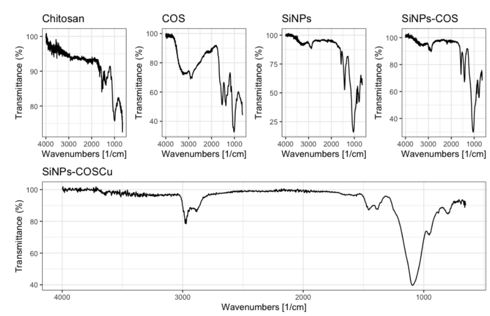


Figure 3. IR spectra of chitosan, COS, SiNPs, SiNPs-COS, and SiNPs-COSCu.

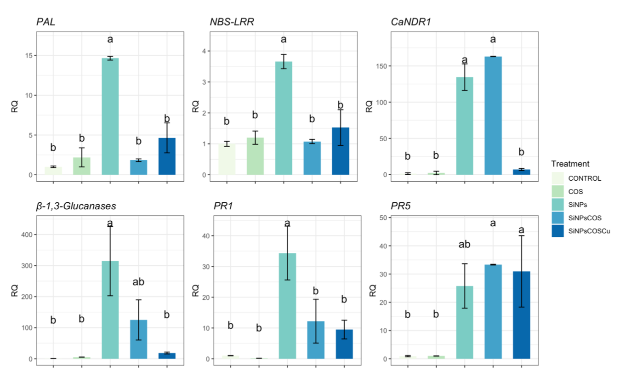


Figure 4. Molecular responses of *Coffea arabica* var Bourbon plants to foliar exposure to distilled water (control), COS, SiNPs, SiNPs-COS and SiNPs-COSCu in greenhouse conditions.

Conclusions

This study shows that functionalizing silica nanoparticles (SiNPs) with chitosan oligosaccharides (COS) and copper ions (Cu^{2+}) enhances both their physicochemical and biological properties. Characterization confirmed improved stability and surface modification, while treated *Coffea arabica* plants exhibited strong induction of defense-related genes, especially with SiNPs-COSCu. These results indicate that functionalized SiNPs effectively stimulate plant immunity, suggesting their potential as sustainable tools for enhancing disease resistance in coffee crops.

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