A NEW COFFEE (Coffea arabica) DEFENSIN (CD1): A BIOTECHNOLOGICAL ALTERNATIVE TO CONTROL ASIAN SOYBEAN RUST (Phakopsora pachyrhizi)

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The Asian soybean rust, caused by the fungus Phakopsora pachyrhizi, is one of the most serious phytosanitary problems of soybean in Brazil. The use of synthetic fungicides is the main method to control, however, these compounds are extremely dangerous to the environment. Alternative methods are continuously under research and development in order to improve phytopatogen control. In this context, this study was carried out to characterize the antimicrobial potential of a new coffee defensin. In the present study, the activity of a new defensin from coffee (Coffea arabica) named CD1 (Coffee defensin 1) was tested against Phakopsora pachyrhizi, which causes Asian soybean rust. CD1 was subcloned into the yeast expression vector pGAPZ\textsuperscript{α}-B\textsuperscript{®} in two recombinant forms, one of them with a his-tag at C-terminal end (CD1tC), and the other with a his-tag at N-terminal end (CD1tN). Both forms of cd1 were expressed in bioreactor and recombinant proteins were by using Immobilized Metal Affinity Chromatography associated to reversed - phase HPLC chromatography (C18). The antifungal activity of rCD1 (rCD1tC and rCD1tN was carried out in two assays. The first assay to analyze inhibition of the P. pachyrhizi uredospore germination and second assay to analyze the disease severity caused by P. pachyrhizi in planta. rCD1tC at 3 \textmu g/\textmu L inhibited 26\% of in vitro uredospore germination after 16h incubation, while rCD1tN inhibited 18\% of in vitro uredospore germination. Additionally, the two recombinant forms also decreased the severity of Asian rust, as demonstrated by in planta assay. rCD1, at 3 \textmu g/\textmu L, resulted in no disease as compared to 80\% rust severity in absence of the recombinant protein 14 days post inoculation. To our knowledge, this is the first report of a coffee defensin active against phytopathogenic fungus Phakopsora pachyrhizi. These results revealed the potential of rCD1 as a candidate to be used to control Asian Soybean Rust.

Key words: Coffee Defensin, Pichia pastoris, Phakopsora pachyrhizi

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