Purification, biochemical characterization, and antimicrobial activity of a new lipid transfer protein from *Coffea canephora* seeds

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**ABSTRACT.** Coffee, an agronomical crop of great economic
importance, is also among the most commonly traded commodities in worldwide markets. Antimicrobial peptides, which play a role in plant defense, have been identified and isolated particularly from seeds. We isolated and immunolocalized Cc-LTP2, a new lipid transfer protein (LTP) from Coffea canephora seeds. We report its antimicrobial activity against various phytopathogenic fungi of economic importance, and against the bacterium Xanthomonas euvesicatoria. Peptides from C. canephora seeds were initially extracted using acid buffer and subjected to ion-exchange and reverse-phase chromatographies. A purified peptide of approximately 9 kDa, which we named Cc-LTP2, was then subjected to amino acid sequencing. The analyses showed that it was similar to LTPs isolated from various plants. The tissue and subcellular localization of C. canephora LTPs indicated that they were located in cell walls and intracellular palisade parenchyma, mainly in large vacuoles. The results of immunohistochemistry and histochemistry superposed from C. canephora seed tissues showed that LTPs and lipid bodies are present in organelles, supporting the hypothesis that LTPs from seeds are involved in lipid mobilization during germination. Cc-LTP2 did inhibit the development of the phytopathogenic fungi Colletotrichum lindemuthianum, Colletotrichum gloeosporioides, Fusarium solani, Fusarium lateritium, and Colletotrichum sp, but did inhibit X. euvesicatoria. Cc-LTP2 also increased membrane permeability and induced endogenous production of reactive oxygen species in all the fungi tested.

Key words: Antimicrobial peptides; Phytopathogenic fungi; Chromatography; Membrane permeabilization; Reactive oxygen species