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Rayssa Almeida Garcia, Gillet F X, Macedo L L P and Grossi-de-Sa M F
[1]University of Brasilia, Brazil
[2]Embrapa Genetic Resources and Biotechnology, Brazil
[3]Catholic University of Brasilia, Brazil

Gene silencing through RNA interference as a biotechnological approach for the control of crop insect-pests have been intensively applied in the last few years. dsRNA microinjection and in vitro feeding are the most wildly used approaches for administering RNAi in insects. However, RNAi e ciency appears to be variable among di erent insect groups, especially when applied by feeding, for some insect groups the oral delivery of the dsRNAs has been reported highly ine ective. In initial studies, our gene silencing data for cotton boll weevil (Anthonomus grandis) were unclear when dsRNA administration was done by feeding. e purpose of this work was to assess the possibilities of RNAi as a tool for the control of this insect pest using oral delivery of dsRNAs and to investigate the reason for the low e ciency in gene silencing, aiming to develop a strategy to deal with the e ciency and usage of dsRNA by feeding. Data showed an optimal nucleasic activity of the A. grandis gut nucleases at acid pH, ranging from 5.5 to 6.5 and the A. grandis gut homogenate signi cative degraded both dsRNA and dsDNA. ree nuclease sequences were found in A. grandis transcriptome, named AgNuc1, AgNuc2, and AgNuc3 in which AgNuc2 and AgNuc3 showed to be highly expressed in the insect gut. e silencing of the three nuclease genes strongly diminished dsRNA degradation when dsRNA were incubated with homogenate from silenced insects. On the other hand, when dsRNAs were protected with a Cell Penetrating Peptide (CPP) fused with a dsRNA Binding Domain (DRBD), no dsRNA degradation was found. Furthermore, dsRNAs complexed with CPP-DRBD were found to enter into A. grandis gut cells. e dsRNA complexed administered in the diet caused a greater gene silencing, compared to naked dsRNA. All data point out to the relevance for overcoming the gut nucleases with/or in parallel with the RNAi applications for the control of crop insect-pests.

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Rayssa Almeida Garcia is currently pursuing PhD in Molecular Biology from the Federal University of Brasilia, Brazil. She does her research work in the Plant-Pest Interaction Laboratory at Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil under the supervision of Dr Maria Fatima Grossi-de-Sa. She has published a large number of papers in reputed journals and is a Fellow Member of the World Academy of Science.

rayssaag@gmail.com

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