



XXVII Congresso Brasileiro
X Congresso Latino-Americano

Entomologia

Saúde, Ambiente e Agricultura

02 a 06 de setembro de 2018, Expogramado, Gramado/RS

Anais

Promoção e realização



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Increase in the efficacy of gene silencing using viroid-like dsRNAs in the insect pest cotton boll weevil

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Nowadays RNA interference (RNAi) is being used to control insect pests, which came as an alternative applied to GMO crops. The RNAi mechanism is demonstrated to be efficient against several insect pests through oral delivery; while for other insects, including the cotton boll weevil (CBW), *Anthonomus grandis*, it is unsuccessful. The weak or non-response to silencing may be explained by different factors, including the presence of insect gut nucleases, which can cleave the dsRNA into siRNAs or completely degrade it. Another difficulty in insect pest control through RNAi oral delivery is the plant own RNAi machinery that can cleave dsRNAs into siRNAs indiscriminately. Face to this barrier, this study aims the use of a new strategy, based on viroid-like dsRNAs, to manage CBW through RNAi. Viroids are structured RNAs resistant to plant nucleases, and can be addressed to chloroplasts, where there is no RNA machinery. The gene of interest, chitin synthase II, was inserted in a viroid sequence and the dsRNA was synthesized. Data showed that the synthesized viroid-like dsRNA increased gene silencing in CBW in three times, causing a raise in mortality of 65%. Also, it decreased oviposition in female insects in 50%, as well as 60% in egg viability, when compared to linear dsRNA. Also, confocal microscopy showed that labeled viroid-like dsRNA was localized in chloroplasts of *Arabidopsis thaliana*, while labeled linear dsRNA was diffused. The presented data are promising since the dsRNA is addressed to chloroplasts, disrupting or decreasing its degradation by plant nucleases. Also, the viroid-like dsRNA shows a higher effect in bioassays with CBW, making it a better choice to control this insect pest. Further, this structured dsRNA can be coupled with other insect control strategies, such as nanoparticles

Palavras-chave: RNA interference; insect controle; viroid structure

Apoio institucional: CAPES, UNB, EMBRAPA CENARGEN, FAPDF

Filiação institucional: Universidade de Brasília/ Embrapa Cenargen