Engineered Cry8H toxins with activity towards to the cotton boll weevil, *Anthonomus grandis*, (Coleoptera: Curculionidae).

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The Cry proteins are δ-endotoxins produced in crystal form of proteins by *Bacillus thuringiensis* strains during the sporulation process. These proteins show high specificity and toxicity to certain classes of insects and invertebrates, which increased the interest for the search of new genes encoding active molecules to target insect-pests. Among the insect-pests that attack cotton crops, the cotton boll weevil, *Anthonomus grandis*, is one of the most important in Brazil. Due to its endophytic behavior, its control with chemical insecticides is not efficient and expensive, being also associated with many health and environmental problems. In this context, we developed new sequences of *cry8H* that were specific and more effective against the cotton boll weevil, using techniques such as DNA Shuffling and Phage Display. The *cry8H* sequence was fragmented and randomly recombined, thus obtaining the shuffled genes, generating a combinatory library of mutant genes *cry* with 10^5 transformants. The created mutants were screened by Phage Display using *A. grandis* border membrane vesicles (BBMV), in which the potential receptor of the toxin is present. The clones were further selected through bioassays to validate their insecticidal activities. These clones were sequenced to confirm the mutations and 4 clones exhibited 2-6 fold enhanced insecticidal activity compared to the wild type gene. The efficiency of the recombination by DNA Shuffling and Phage Display to generate new active molecules of Cry8H against *A. grandis* is discussed.

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