

Insecticidal Activity of Shuffled Alpha-Amylase Inhibitors

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Transgenic crops have been used in agriculture to control insect pests. Although the most common strategy is to produce an insecticidal *Bacillus thuringiensis* delta-endotoxin from an introduced gene, the use of other insecticidal proteins, stacked genes or fusion proteins is also important for preventing development of pest resistance. Earlier it was found that the seeds of pea plants expressing a gene for alpha-amylase inhibitors (α AI) from the common bean (*Phaseolus vulgaris*) were protected from damage by bruchids through inhibiting the insect alpha-amylase enzymes. We have now designed a strategy to improve α AI molecules to confer higher and specific activity against target insects. Our studies focused on the phage-display screening of α -amylase inhibitors by using a 10^7 recombinant α -amylase inhibitors library produced by *in vitro* recombination (DNA shuffling) of homologous α A11 and α A12 genes. Several different mutants were selected using affinity for α -amylase enzymes from both *Zabrotes subfasciatus* and *Anthonomus grandis* insect pests. The different mutants were sub-cloned in plant expression vectors and introduced into *Arabidopsis thaliana* plants. This represents an important step to guarantee the correct folding needed for the activity of these inhibitor variants. The transformed plants were used to evaluate, *in vitro* and *in vivo*, toxicity against those insect pests by bioassay. Our data indicate variability among the toxicity of the recombinant proteins. We interpret the results as showing that the novel α AI genes have potential for use in gene constructions, such as pyramiding of genes encoding for different insecticidal molecules, as an alternative method to preventing the development of pest resistance, and for conferring greater levels of pest control. In additional, this study documents that DNA shuffling and phage-display combined techniques are important strategies to generate large number of mutants *in vitro* and to identify new molecules with improved activities towards insects of different orders.

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