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PROTEOMIC TO ANALYSE PROTEOLYTIC CHANGES IN *HELICOVERPA ZEA* GUTS UPON FEEDING ON PROTEASE INHIBITOR CONTAINING DIET

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Pest insects like Helicoverpa species are a polyphagous pest of many important crop plants throughout the world, responsible for heavy economical losses. Chemical control of H. zea insects is often not effective, as they are able to develop the resistance to chemicals like DDT, organophosphates and pyrethroids. Proteolytic activities in the larvae guts have been investigated and shown to be due predominantly to extracellular serine proteinases of trypsin and chymotrypsin type.

One of the plant natural defence mechanisms against insect pest is inhibition of digestive proteinases by proteinase inhibitors (PIs). The resulting deficiency in free amino acids causes developmental delays, and in some cases, mortality of larvae. PIs have been shown to restrict the growth and/or the development of herbivourous insects either when added to artificial diets or when expressed in transgenic plants. However some insects, including Helicoverpa, are able to overcome the PIs in their natural or enginereed diet by up-regulating a set of "insensitive" proteinases.

In this report, analyses on the proteolytic content of H. zea guts after feeding of larvae on control and SKTI (Soybean Kunitz Trypsin Inhibitor) containing diets are reported. Isolation of H. zea proteinases was carried out by affinity chromatography through a sepharose-CNBr column on which the mustard trypsin inhibitor MTI-2 had been immobilised. Isolated proteinases were characterised by activity gels, SDS PAGE and iso-electric focussing and their interaction with substrates. The effect of plant proteinase inhibitors on the isolated proteinases was also tested. Isolated enzymes were partially sequenced by tandem mass spectrometry. Polypeptide sequences, matched with available cDNA sequences, allowed the assignment to specific trypsin and chymotrypsin genes of Helicoverpa species.

Proteinase identified in this study would be good candidates for further interactions studies with PIs to clarify structural reasons of proteinase inhibitor insensitivity.