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**Poster Abstract – C.44**

# CHARACTERIZATION OF PROTEASE INHIBITORS SELECTED FROM A COMBINATORIAL LIBRARY AGAINST AN INSECT INSENSITIVE PROTEASE

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Insect digestive proteases are the target of specific plant protease inhibitors (PIs). PIs are small proteins that, acting as a substrate, form stable complexes with proteases, strongly reducing their activity. Inhibition of gut proteases causes severe retardation in growth and development of insect larvae. Transgenic plants expressing heterologous PIs, have been demonstrated effective in reducing growth and diffusion of insect larvae. Anyway, in the last years, it became evident that insects can adapt their gut enzyme content upon ingestion of PIs, by producing new and "insensitive" proteases. On the other hand, also the PI content of plants consists of several inhibitors, presumably with different specificities. For both fundamental and applicative studies, the co-evolving plant PIs and insect proteases system shows interesting features to be studied.

In the work presented in this communication, affinity chromatography was used to isolate a trypsin-like protease ($P_{Hz}$) from *Helicoverpa zea* larvae raised on a PI-containing diet. The isolated trypsin was found to be insensitive to four different plant protease inhibitors and used as target for a "phage display" based selection of MTI2 variants. MTI-2, Mustard Trypsin Inhibitor 2, belongs to a trypsin inhibitors family found only in *Cruciferae*, which does not show any structural homology with other plant PIs. A MTI-2 phage display library was then constructed by randomizing codons corresponding to five aminoacids around the P1 reactive site ($R^{20}$): $A^{18}P^{19}R^{20}I^{21}F^{22}$. A library of $9.3x10^7$ independent colonies was obtained. A previous phage display selection of the MTI-2 library against immobilized bovine chymotrypsin allowed to isolated active variants effective in reducing life span and vitality of aphid species *Acyrthosiphon pisum* [1].

The selection of MTI2 variants by screening against the $P_{Hz}$ trypsin led to the identification of two variants with the sequences: $K^{18}N^{19}R^{20}L^{21}S^{22}$ and $T^{18}P^{19}L^{20}T^{21}A^{22}$. The two mutants were produced as recombinant proteins by using the *Pichia pastoris* expression system, and checked by N-terminal sequencing. Activity analysis against bovine trypsin or chymotrypsin, and against the isolated $P_{Hz}$ protease showed a complete inactivity of the two recombinant inhibitors.

In order to understand whether structural constraints in the novel inhibitors could affect their activity, circular dichroism analysis and site specific back mutations are in progress.

[1] Ceci L.R., Volpicella M., Rahbé Y., Gallerani R., Beekwilder J and Jongsma M.A. (2003) *Selection by phage display of a variant mustard trypsin inhibitor toxic against aphids*. Plant Journal 33, 557-566.