

## NOVEL STRATEGIES TO ENHANCE PLANT DEFENCE AGAINST INSECT PEST

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The insecticidal proteins delivery to herbivorous target organs is one of the primary issue for the development of novel biopesticides more selective and safe.

This work is focused on the study of molecules affecting the permeation of the insect peritrophic membrane as the AcMNPV ChiA (Rao *et al* 2004; Corrado *et al* 2007) and the production of fusion proteins carrying specific domains (CPPs) allowing the internalization of molecules into the cells. The CPP used in my research activities is HIV-1 TAT transduction domain, fused with the eGFP (Caron *et al.*, 2004).

The **ChiA** recombinant protein and the **eGFP** and **TAT-eGFP** chimeric proteins were successfully purified from different biological systems, transgenic tobacco plants and *E. coli*, respectively. The viral ChiA retains its activity when expressed in plant and this activity is stable at different pH values. The purified recombinant ChiA, eGFP and TAT-eGFP proteins were tested to evaluate the efficiency of permeabilization of the peritrophic membrane to molecules of different sizes and structure and for efficiency in internalization in insect gut epithelial cells, respectively. The obtained results demonstrated that the ChiA protein alters the permeability of *Bombyx mori* peritrophic membrane to methylene blue. This is likely the consequence of the reduced integrity of the membrane treated with the recombinant enzyme. Moreover our data showed that the TAT-eGFP protein is internalized into *Bombyx mori* columnar cells more efficiently than the eGFP protein. Encouraged by this result, new recombinant proteins fused to TAT domain will be produced and tested for internalization into epithelial gut cells of lepidopteran larvae. Furthermore, the transgenic tobacco ChiA-mycKDEL9 line, which expresses the ChiA gene, was crossed with a transgenic tobacco line expressing a hormonal insect peptide with pesticide activity, **TMOF** (Tortiglione *et al.*, 2002). *H. virescens* larvae fed with tobacco hybrids co-expressing the **ChiA** gene and the **polyTMOF** synthetic gene showed higher mortality than larvae fed with parental lines indicating an additive effect of the two genes. The recombinant ChiA protein is being tested *in vitro* in combination with the TMOF peptide to elucidate if the ChiA protein enhances the uptake of the peptide in *H. virescens* larvae.

In addition, the Bowman–Birk serine proteinase inhibitor (BBI), a well known antinutritional factor affecting animal digestion (Ryan 1990), isolated from *Lens culinaris* subsp. *culinaris*

(Sonnante *et al.* 2005) was expressed in tobacco plants to monitor its effect on lepidoptera larvae development alone and in combination with other pesticide genes.