Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - A.05

ASSESSMENT OF PVX AS A SYSTEM FOR RECOMBINANT PROTEIN PRODUCTION IN PLANTS

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PVX, recombinant protein, transient expression, Nicotiana benthamiana

Expression systems based on plant viral vectors are beginning to have major impact for the production of specialty products, for the delivery of therapeutics and as a powerful laboratory tool because of their simplicity and rapidity. PVX is one of the most used RNA-viral vector and we employed it for the transient expression of several proteins, differing in their molecular weight and subcellular localization: nef, proinsulin, GAD65, interleukin-10 and expansin.

The HIV-1 *nef* gene product is a 27kDa cytosolic protein critically important for immunodeficiency-virus replication and disease progression *in vivo* and it is highly conserved both in human and simian Virus Strain; IL-10 is a 18.5kDa contra-inflammatory secreted cytokine with potential application in the treatment of autoimmune diseases; proinsulin is a cytosolic 9.4kDa protein produced by the pancreatic beta-cells and serves as the precursor molecule of insulin; GAD65 is a 65kDa membrane-anchored protein involved in the development of autoimmune diabetes; expansin is a 27kDa cell-wall plant protein which is involved in cell-wall enlargement.

We realized two PVX expression vectors by the GATEWAY technology and used them for cloning the molecules described. Further more, murine IL-10 and human GAD65 were engineered for targeting to different subcellular compartments. To determine the stability of the inserted genes, for each construct we performed subsequent cycles of infection of *N.benthamiana* plants and analyzed them by RT-PCR. Furthermore, we performed protein expression analysis to assess recombinant protein accumulation. The stability of the PVX expression vector and the protein expression level depending on the length of the inserted genes and on the protein subcellular localization are discussed.