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Poster Abstract – A.06

PRODUCTION OF TRANSGENIC TOMATO PLANTS EXPRESSING THE HIV-1 *GAG* GENE

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The improvement of techniques for genetic manipulation of plants has recently allowed the production of commercially valuable and clinically important recombinant proteins, either in cells cultured *in vitro* or in plants grown *in vivo*. The latters could also provide a simple and inexpensive delivery vehicle to produce an oral vaccine, which offer several practical advantages specially for developing countries. Among crops, tomato is a good candidate for the production of an oral vaccine that can be delivered after simple and inexpensive processing of raw material.

HIV, the retroviral agent of the "Acquired ImmunoDeficiency Syndrome" (AIDS), is presently controlled by anti-retroviral therapeutic strategies (HAART). One alternative strategy relies on the development of preventive anti-HIV vaccines. One of these vaccines is based on the capsid protein Gag to induce a strong humoral and cellular immune response.

A binary vector (pEF1) containing a polycistronic sequence, including structural *gag-pol* genes and the regulatory *nef* gene of HIV-1, under the control of $35S^2$ CaMV promoter, was used to transform tomato plants with *Agrobacterium tumefaciens*. Regenerated shoots were selected on rooting medium in the presence of Kanamycin. All rooted shoots were analysed by PCR to screen for the presence of both *gag* and *nptII* genes. These analyses showed that the two genes were co-integrated in 30 out of 49 *npt*II positive plants (61%). *Gag* expression tested by RT-PCR analysis indicated that *gag* gene was transcribed in all plants albeit at different levels. However, preliminary Western analyses with polyclonal human antisera from AIDS patients did not show any Gag protein accumulation in those plants. Previously, only low level of Gag protein was detected in transgenic tobacco plants transformed with the same construct.

In order to check whether the low expression observed in above-mentioned experiments was due to the co-integration of *gag-pol-nef* genes, a series of constructs was made by cloning only the *gag* gene under the control of *Rbc*S promoter and 5'UTR, along with specific signal sequences for protein accumulation in different cellular compartments (cytosol, apoplast, endoplasmic reticulum, chloroplast, and mitochondrion) and *myc*/6xHis tags. Such vectors were used in transient Agroinfiltration assays on tobacco plants, but again no Gag protein was observed so far. On the other hand, when the same vectors were used with the *gus* reporter gene, protein accumulation and gene expression were observed in Western analyses carried out 3 days post infiltration and in hystochemical or fluorimetric assays. Our results were recently confirmed by other groups (Meyers et al. 2005, PBVA, Prague), who in transient experiments detected limited expression only when a *Nicotiana* codon-optimised $Pr55^{gag}$ was used.

Work is in progress in our laboratory to test optimized sequences as well as the effect of different regulatory signals and of gene silencing suppressors (e.g. p19, HcPro) on gene expression and protein accumulation.