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IMPROVEMENT OF THE HIV-1 PR55^{GAG} POLYPROTEIN ACCUMULATION IN TRANSPLASTOMIC PLANTS BY N-TERMINAL TRANSLATIONAL FUSION WITH THE RBCL PROTEIN

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Human immunodeficiency virus type 1 (HIV-1) is the causative agent of AIDS, one of the deadliest infectious diseases in the world. Great interest has been observed in using HIV-1 Pr55^{gag}, the major structural protein, as a candidate protective vaccine eliciting both humoral and cellular immune responses. The Pr55^{gag} polyprotein precursor is processed by the viral protease into four distinct domains, that participate in the production of HIV-1 mature particles. Although, in the last few years, plants have been increasingly explored for the production of biomedicines and vaccine components, the HIV-1 full-length precursor Pr55^{gag} has never been expressed in plants.

In previous study, we expressed the Pr55^{gag} polyprotein either by stable nuclear transformation with protein addressing into plastids or by plastid transformation. Although we demonstrated Gag expression with both technologies, we obtained low protein yield. The transgenic plants accumulated Gag polypeptides up to 0.1% TSP, whereas the transplastomic plants up to 0.5% TSP.

In this study we improved the Pr55^{gag} protein accumulation producing a new vector (pNS40) for plastid transformation. In the pNS40 vector, the *gag* coding region was expressed by the strong *rrn* promoter fused with the 5' translation control region (TCR) that includes the 5'-UTR and 42 N-terminal nucleotides of the *rbcL* open reading frame, and the plastid *rbcL* gene 3'-UTR. Northern analysis carried out on homoplasmic NS40 plants detected two transcripts of about 2 and 3.3 kb in plants, corresponding to mono- and readthrough dicistronic mRNAs, respectively. The presence of the Gag protein in transplastomic plants was analysed by western analysis. The polyclonal HIV-1/p24 antiserum revealed the presence of a strong signal for the polypeptide of about 41 kDa and weaker signals of about 48 and 55 kDa, corresponding to the expected Pr55^{gag} polyprotein and processed products. Capture ELISA demonstrated a protein yield up to 7% TSP (338 mg/kg fresh tissue), 25-fold higher compared to what obtained with previous transplastomic plants.

Further, we examined the Pr55^{gag} protein stability in soil-grown mature plants at both RNA and protein level using leaves of different ages. Compared to *in vitro* plants, Western analyses showed a selective reduction of the 55 kDa band along with an increase of the 24 kDa signal in youngest leaves, the 41 kDa one remaining largely constant, whereas a more general reduction of all polypeptides was observed in older leaves. In general, a higher stability of the recombinant proteins, especially of the intermediate 41 kDa one, could be observed in pNS40 than in previous transplastomic plants, suggesting that the regulatory sequence of the pNS40 vector not only increased the overall protein accumulation, but also protected the recombinant Gag proteins from degradation in older leaves of mature plants.