OVER-EXPRESSION OF DnaK, DnaJ, OsOCI, StPCI, AtAVP1 AND AtHSD1 FOR A MORE EFFICIENT PRODUCTION OF RECOMBINANT PROTEINS IN *Nicotiana*.

Victor Vázquez-Pérez and Miguel Gómez-Lim; CINVESTAV, Genetic Engineering Department; Irapuato, Guanajuato, México. 36821; vvazquez@ira.cinvestav.com.mx.

Key words: recombinant proteins, chaperones, protease inhibitors, virus-like particles.

Introduction. Plants are gaining popularity and acceptance as bioreactors for the production of pharmaceuticals compounds in recent years due to multiple advantages, such as lower production costs, easy scaling up or down and reduction of pathogen contamination risks, among others [1]. However, there are some obstacles that limit the system like low yield, proteolitic activity *in planta*, misfolding of the recombinant proteins, etc.

Several strategies have been tested to overcome these obstacles. For instance, there are reports where the usage of chaperones has ensured the correct folding of recombinant proteins [2], also the co-expression of protease inhibitors to reduce the endogen proteolysis has been tested [3]. On the other hand, it is important to reduce somehow the stress caused by over-expression of the heterologous compounds.

In this project we aimed to enhance the production of recombinant proteins in plants through the stable transformation of *Nicotiana* with chaperones (DnaK and DnaJ), protease inhibitors (OsOCI, StPCI), and stability/anti-stress genes (AtAVP1, AtHSD1)

Methods. To accomplish the objective of this project, the six previously mentioned genes will be cloned in tandem in a plant expression vector constructed in our laboratory to transform *Nicotiana* by *Agrobacterium tumefaciens*. Insertion of the genes into the plant genome will be verified by molecular methods (PCR and Southern Blot), by the expression of the *uidA* (GUS) gene, as well as by the functionality of the gene products via proteases inhibition assays, growth and development measurements, and tolerance to different kind of stresses. Subsequently, some recombinant proteins previously tested in our laboratory, the L1 protein (the main protein of human papillomavirus capsid), and the proteins core, E1 and E2 (from the Hepatitis C virus) will be transiently expressed in the transgenic plants. To compare the yield of the transgenic versus the wild type plants, the virus-like particles (VLP's) will be extracted, quantified, and folding and assembly will be determined.

Results. So far, the six genes fused to several plant linkers have been successfully cloned into the plant expression vector (Fig. 1a), this was verified by restriction mapping and by the amplification of each gene by PCR (Fig. 1b). *Nicotiana* explants (leaf segments) were transformed with this construct and seedlings have been regenerated, rooted and acclimated in soil. Several transformed lines have tested positive to GUS staining.

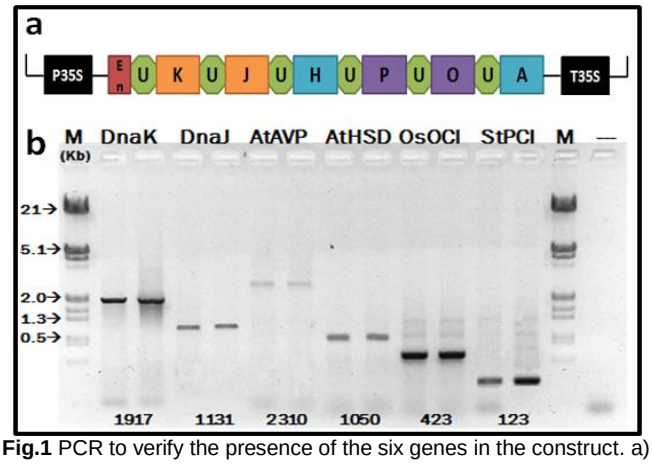

Fig.1 PCR to verify the presence of the six genes in the construct. a) P35S. 35S Cauliflower Mosaic Virus (CaMV) Promoter; En. TEV Enhancer; U. Plant linker; K. DnaK gene; J. DnaJ gene; H. AtHSD1 gene; P. StPCI gene; O. OsOCI gene; A. AtAVP1 gene; T35S. CaMV polyadenylation signal. b) Amplification of each gene (and a duplicate) from the vector. M. Molecular weight marker; --. Negative control. The weight of each gene is shown in the bottom part.

Conclusions. We have generated transformed plants containing the six transgenes. In total the length of the insert was 8447 bp. The plants were grown in the presence of 150 mg/L kanamycin. The plants have tentatively been confirmed as transformed by GUS assays but molecular tests (Southern blot, PCR) are under way to confirm this result. Experiments of transient expression are also in progress to determine whether the expression of the six genes enhances yield and quality of the recombinant proteins.

Acknowledgements. The authors are grateful to CONACYT (262803) and CINVESTAV for the financial support.

References.

1. Gómez-Lim, M. (2011). Plants as platform for production of pharmaceuticals compounds. In: *Bioprocess sciences and technology*. Liong, M. Nova Sciences Publishers, Inc., USA. 295-322.
2. Martínez-Alonso, M., García-Fruitós, E., Ferrer-Miralles, N., Rinas, U., Villaverde, A. (2010). *Microbial cell factories.* 9.(64).
3. Pillay, P., Kibido, T., du Plessis, M., van der Vyver, C., Beyene, G., Vorster, B., Kunert, K., Schlüter, U. (2012). *Appl. Biochem. Biotechnol.* 168.(6.):1608-1620.