# ANALYSIS OF THE HUMAN IFN A-2B EXPRESSION IN TRANSPLASTOMIC TOBACCO

Volodymyr Rudas[1], Nadia Zholobak[2], Anastasiya Klebanovych, Anatoliy Shakhovsky[1], Igor Komarnytsky[1], Olga Ovcharenko[1], Mykola Kuchuk[1]

[1] Institute of Cell Biology and Genetic Engineering National Academy of Science of Ukraine,

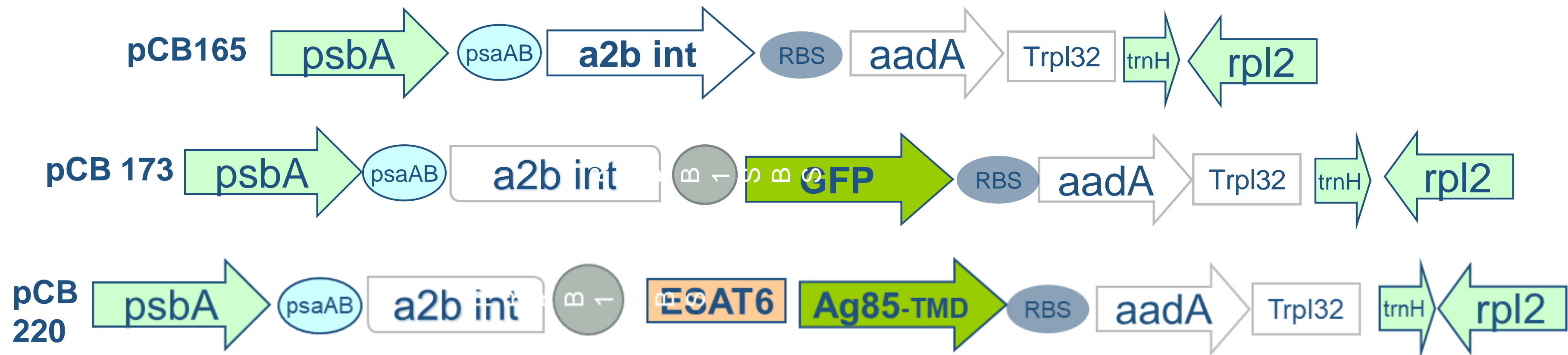
[2] Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine

**Introduction.** Nowadays, plants are increasingly used as effective and cheap platforms for the production of recombinant proteins. The value of plant-based platforms lies in the ability of correct modification of human and animal proteins to gain their functional activity, the absence of contamination of with bacterial toxins and/or human pathogens. Pharmaceutical proteins can be synthesized in plants with genetically transformed plastome in higher concentrations compared to the transgenic due to the presence of extremely increased copy number of genes in each cell (several thousand copies). In addition, plastids are maternally inherited in most crops, which prevents pollen mediated spread of heterologous genes. In molecular farming the chloroplast transformation gives a unique advantage for the development of biopharmaceutical production [Daniell et al., 2016].

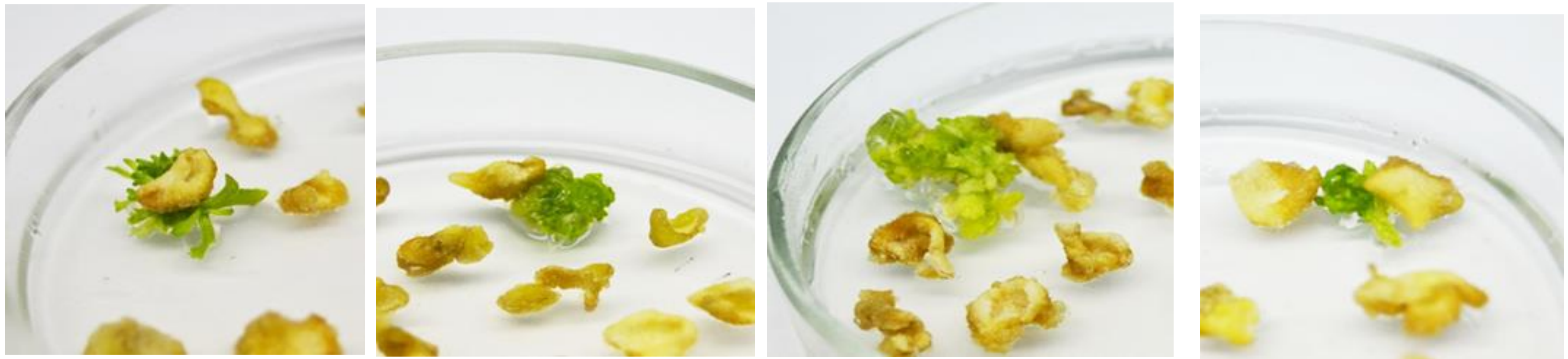
**The goal of this work** was to create biotechnological transplastomic tobacco plants for production of human interferon, the cytokine with antiviral activity; and to analyze the activity of obtained plant-based recombinant interferon against viruses.

**Materials and methods.** Transplastomic tobacco plants were obtained by biolistic transformation with vectors containing the *ifn α-2b* and *aadA* selective genes together or in combination with other genes. The obtained plants were analyzed by the PCR and the hybridological method. The interferon activity was measured by microtitration method (Rubinstein et al. 1981) based on the ability of studied extracts to protect renal epithelial cells MA-104 from *Cercopithecus aethiops* (from cell collection of Zabolotny Institute of Microbiology and Virology, NAS of Ukraine) against cytopathic effect of vesicular stomatitis virus (VSV), Indiana strain (collection of Zabolotny Institute of Microbiology and Virology, NAS of Ukraine)

# *Schematic representation of the vectors*

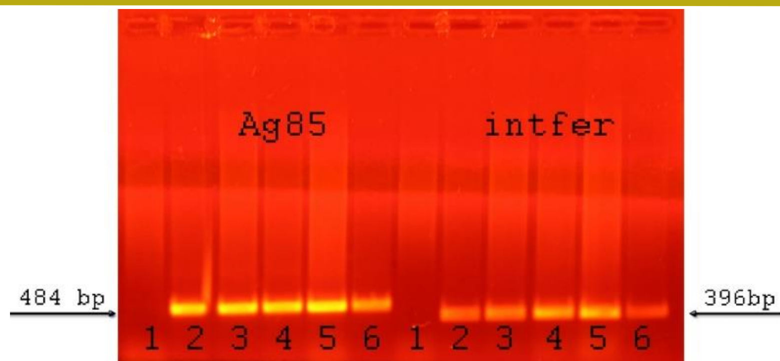



Selection of tobacco calli on the MS regeneration medium with spectinomycin and streptomycin



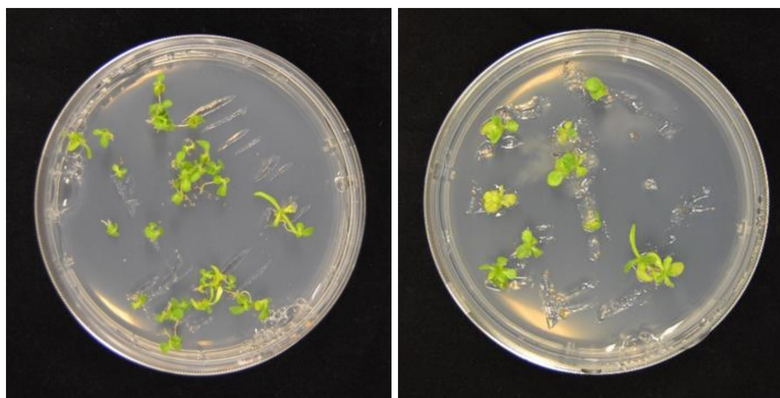
As a results after bombardment and futher selection 12 spectinomycin and streptomycin resistant plants transformed by pCB 165 vector, 7 transformed by pCB 173, and 5 transformed by pCB 220 were produced

# *PCR analysis of N. tabacum lines of the Samsun variety transformed with the pcb220 vector for the presence of genes a2b i fbpB$^{\Delta TMD}$*




PCR analysis of the total tobaccos DNA transformed by vector pCB 220 using primers for *fbpB*$^{\Delta TMD}$ and i *a2b-iinterferon genes*: 2- 6 – transplastomic *N.tabacum*, plants. 1- negative control– non transformed plants *N.tabacum. For a2b interferon gene* 5-ttgatgctcctggcacag -3' and 5-gaggttgtcagagcagaa-3' primers were used for *fbpB*$^{\Delta TMD}$ 5'- tctacagcgactggtacagc-3 and 5'-tcaggttgctgctacgaacg-3 primers were used

## *Hybridological analysis*



Absence of segregation in the T1 generation on the NS selective medium with 400 mg/l spectinomycin and 400 mg/l streptomycin in transplastomic tobacco clones S 554 (pcb 165)–left and S 465 (pcb220)–right.

# Table. Human a2b interferon activity in leaves of T1 generation of transplastomic tobacco.

| Tobacco genotypes | Vector for transformation | Interferon activity, $\times 10^3$ IU/g rawї weight | Interferon activity, $\times 10^3$ IU/mg protein |
|---|---|---|---|
| Samsun (non -transformed) | | 0,00 | 0,00 |
| S 548 $T_1$ N2 (1) | pCB 165 *(aad*A+ *a2b ifn)* | 43,20 | 1,03 |
| S 465 $T_1$ N 1 (3) | pCB 220 (*aad*A+ *a2b ifn +esxA fbpB$^{\Delta TMD}$)* | 6,51 | 0,38 |
| S 519 $T_1$ N 1(4) | pCB 173 *(aad*A+ *a2b ifn+ gfp)* | 0,33 | 0,02 |

**Results.** The integration of genes of interest into the plastome was confirmed by PCR analyses. $T_1$ generation of transplastomic plants demonstrated maternal inheritance of the selective *aadA* gene (coding for resistance to spectinomycin and streptomycin). Transplastomic clone of tobacco, with *aadA* and *ifn α-2b* genes, demonstrated high activity of human interferon α-2b (42,300 IU/g of raw weight). For comparison, the interferon activity in the leaves of the transgenic clone of tobacco was about 1000 IU/g of raw material. We also used constructs in which the interferon gene was fused with some other genes. It was previously demonstrated that some proteins can be unstable in transgenic chloroplasts and their stability can be increased by fusion with other genes. In particular, IFN-γ degraded quite severely in tobacco chloroplasts (< 0.2% of TSP), but after fusion with β-glucuronidase (GUS), its yield significantly increased (up to 7% of TSP) [Leelavathi and Reddy, 2003]. We observed opposite effects in our research. Co-expression in tobacco chloroplasts of *ifn α-2b* gene with *esxA* and *fbpB*$^{\Delta TMD}$ genes, encoding proteins, that induce the immune response to *Mycobacterium tuberculosis* Esat Ag 6, decreased the activity of human interferon α-2b. In plants with *ifn α-2b* and *gfp* fused genes interferon activity was almost absent.

**Conclusion**. Transplastomic tobacco plants producing high amounts of human interferon α-2b were obtained. In our research. Co-expression of *ifn α-2b* gene with other genes in tobacco chloroplasts reduced the activity of human interferon.

**References**

Daniell H., Lin C.-S., Ming Y. *et al.* (2016) Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biology.17 (134):1-29. https://doi 10.1186/s13059-016-1004-2

Leelavathi S. & Reddy V.S. (2003) Chloroplast expression of His-tagged GUS-fusions: a general strategy to overproduce and purify foreign proteins using transplastomic plants as bioreactors. Mol. Breed. 11, 49 -58. https://doi.org/10.1023/A:1022114427971