the 4th international symposium on euroasian biodiversity

PLANT GENETIC MODIFICATIONS AND BIODIVERSITY KEEPING IN ASEPTIC CULTURE CONDITIONS

Prof. Mykola (Nikolay) Kuchuk,
Institute Cell Biology and Genetic Engineering
NASU, Kiev, Ukraine

3 July 2018

www.icbge.org.ua

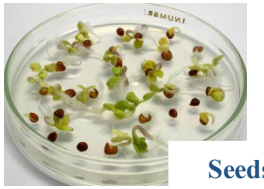
ІКБГІ НАН України

Plant Germplasm Bank of the World Flora

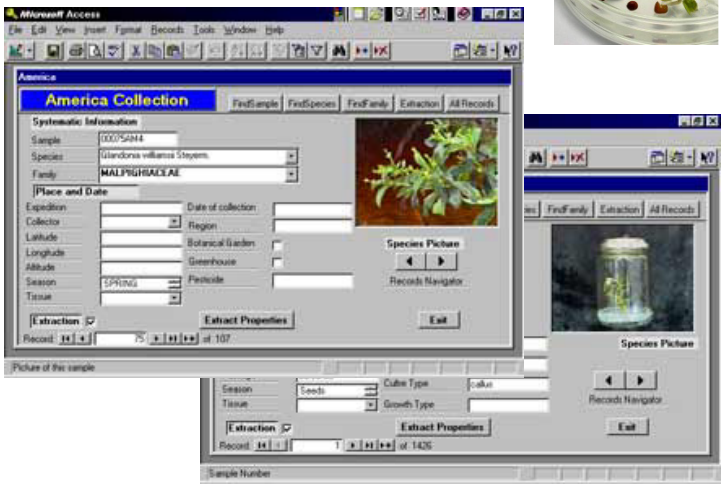
1993 – beginning of the work

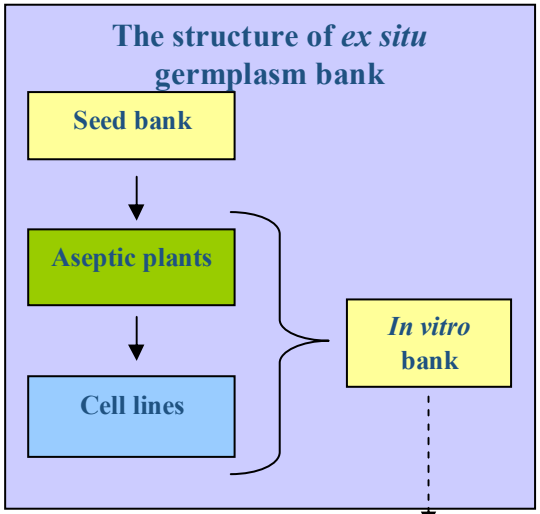
1999 – according to the regulation of the Cabinet Council of Ukraine the germplasm bank was included into the list of objects of the national scientific dignity

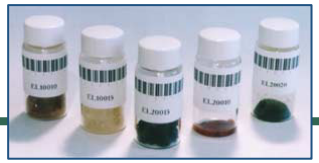
Angiosperms in the germplasm bank		
	Seed bank	*In vitro* bank
Families	166 Monocotyledons – 38 Dicolyledons - 128	117 Monocotyledons – 27 Dicolyledons - 90
Species	3569	2019
Samples (lines)	4315	2416



Seeds →



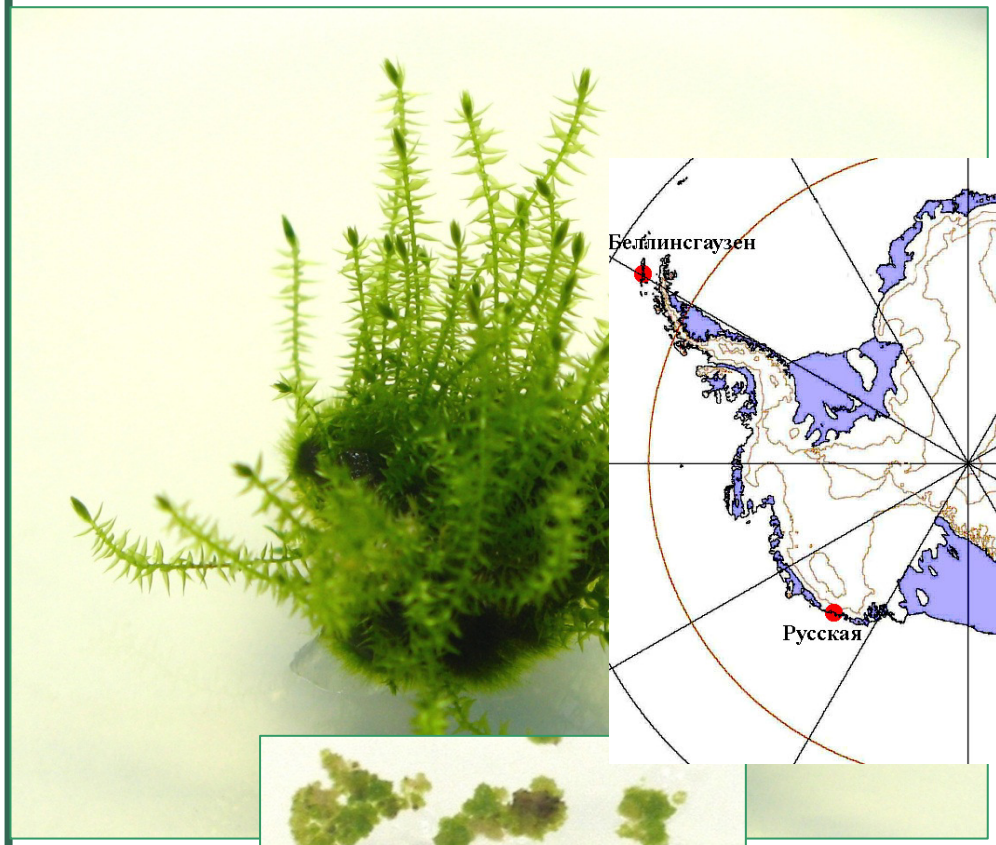


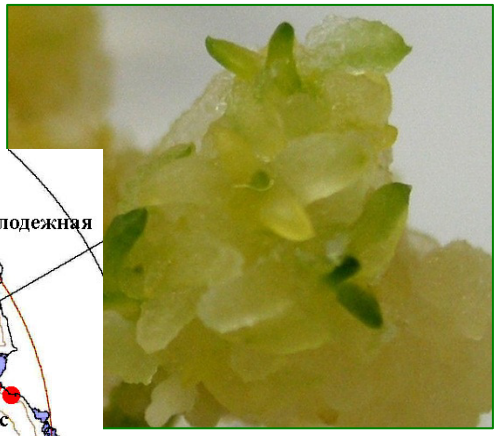


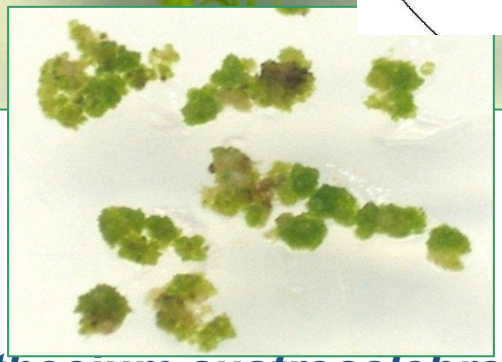
Plant biodiversity conservation: Germplasm bank of world flora

In vitro bank of the world flora:
A – collection of cell lines, B – collection of aseptically growing plants.

Plants of Antarctica in ICBGE in vitro collection





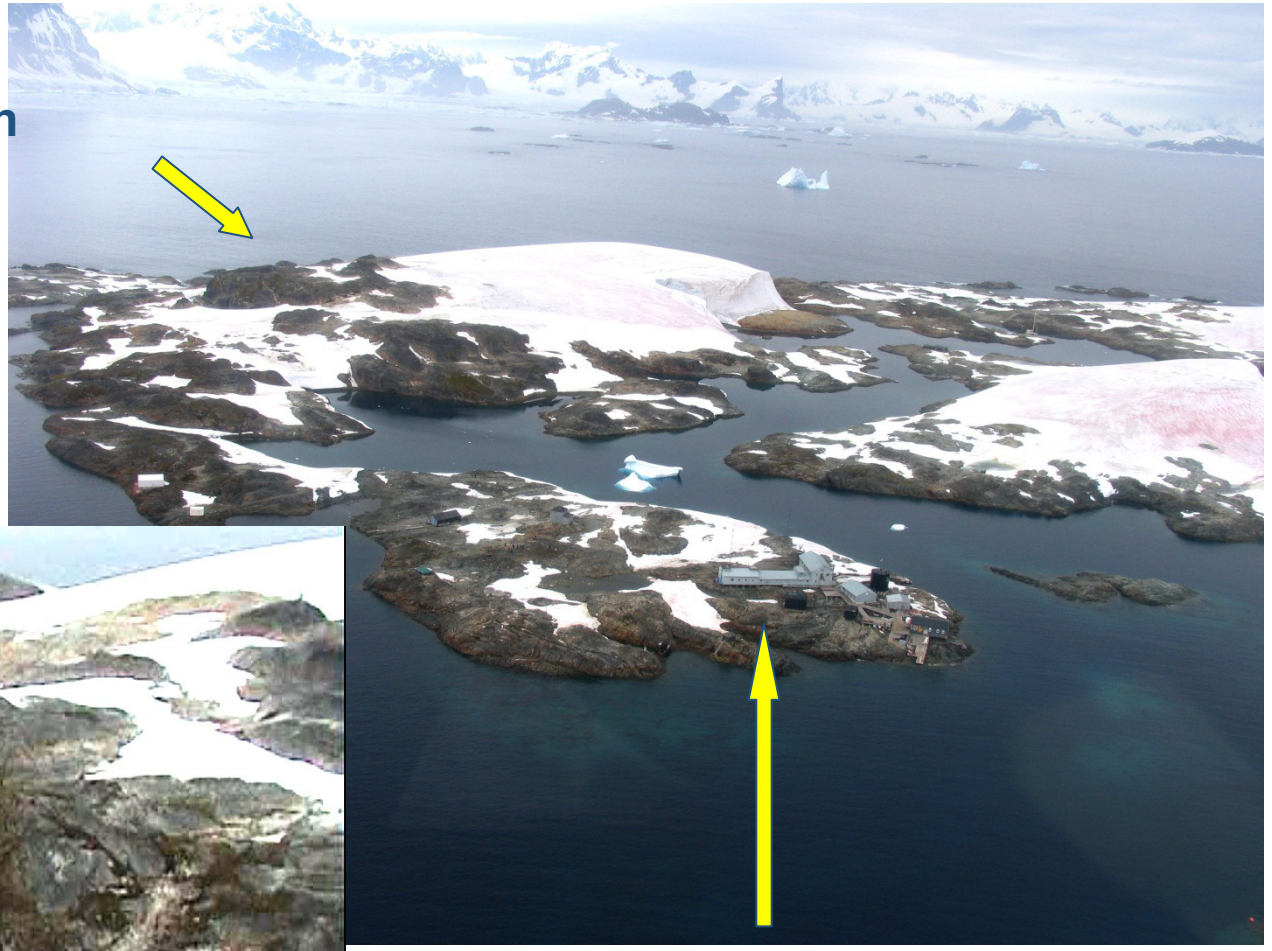


Brachythecium austrosalebrosum

Colobanthus quitensis

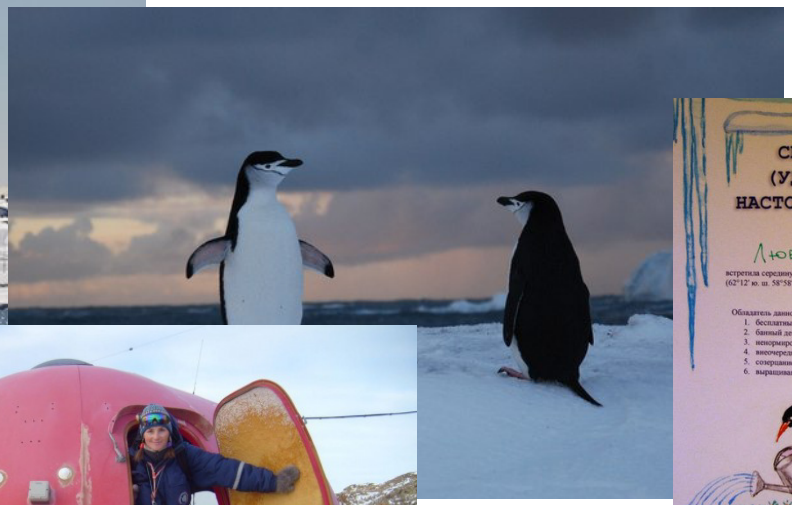
Ukrainian “Vernadsky” station

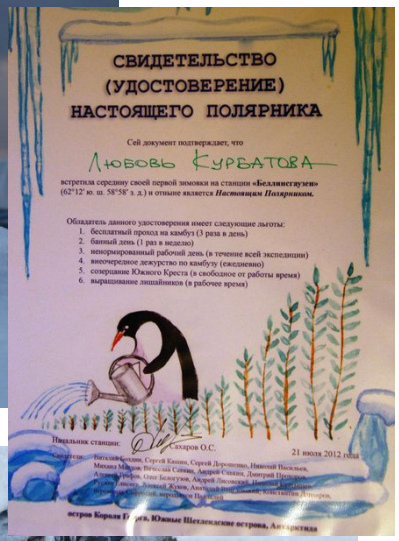
Place of plant collection



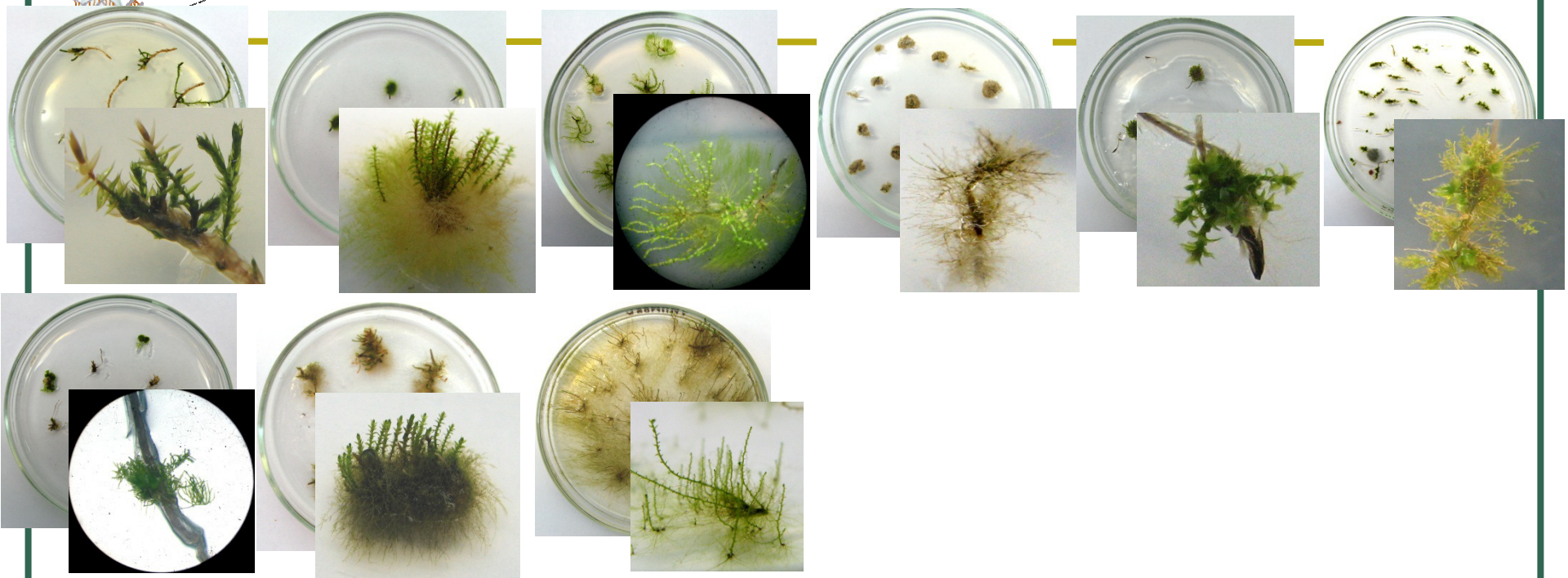
Station

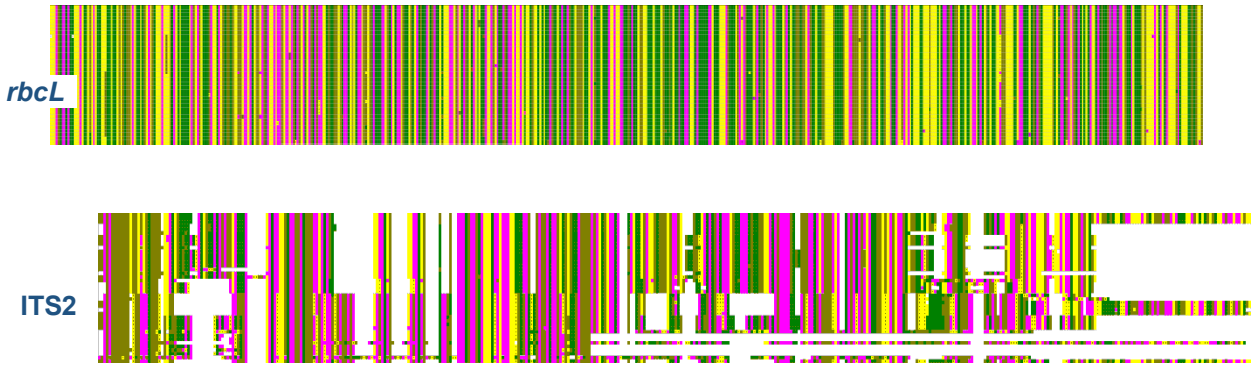
Russian “Bellinsgauzen” station





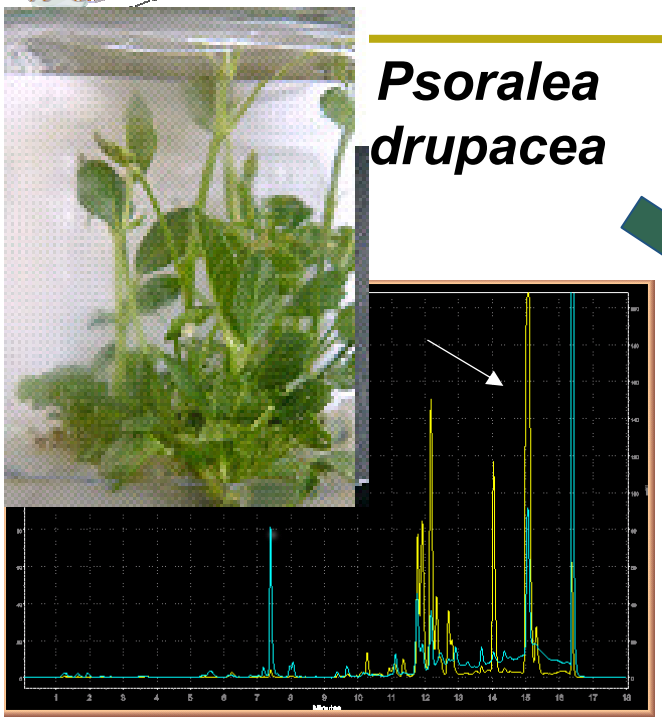
ANTARCTICA MOSSES





Plant bar coding based on nuclear and chloroplast DNA regions (*trnH-psbA, rbcL,* ITS2, ITS) for *D. antarctica, C. quitensis, B. pseudotriquetrum*, B. *archangelicum, W. fontinaliopsis, P. nutans*.

Bakuchiol production in in vitro systems of Psoralea spp.

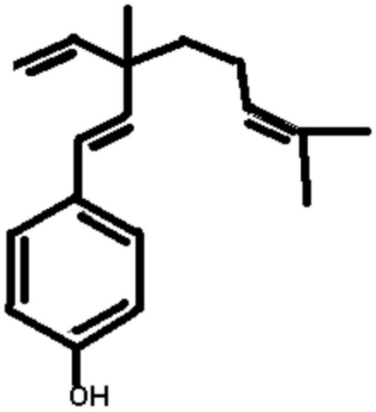


Psoralea drupacea

Up to **11%**

0%

Psoralea drupacea



M320

Up to **0,7%**

0%

Psoralea bituminosa

	BAP, mg/L					
NAA, mg/L		0	0,1	0,5	1	2
	0	0,20	0,07	0,16	0,2	0,15
	0,1	0,31	0,14	0,3	0,27	0,37
	0,5	0,3	0,42	0,21	0,24	0,15
	1	0,16	0,2	0,72	0,24	0,62
	2	0,16	0,44	0,57	0,61	0,7

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 3870−3879

pubs.acs.org/JAFC

Study on Betalains in *Celosia cristata* Linn. Callus Culture and Identification of New Malonylated Amaranthins

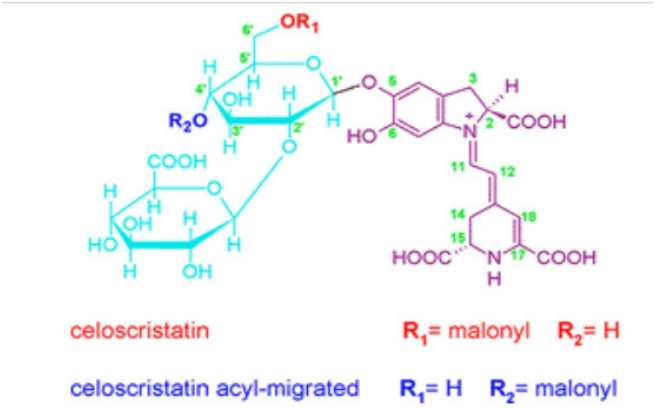
Kateryna Lystvan,[*,†] Agnieszka Kumorkiewicz,[‡] Edward Szneler,[§] and Sławomir Wybraniec[*,‡]

[†]Department of Genetic Engineering, Institute of Cell Biology and Genetic Engineering of National Academy of Sciences of Ukraine (NASU), Academika Zabolotnoho, 148, 03143 Kyiv, Ukraine

[‡]Department of Analytical Chemistry, Institute C-1, Faculty of Chemical Engineering and Technology, Cracow University of Technology, ul. Warszawska 24, Cracow 31-155, Poland

[§]Department of Chemistry, NMR Div, Jagiellonian University, ul. Ingardena 3, 31-007 Cracow, Poland

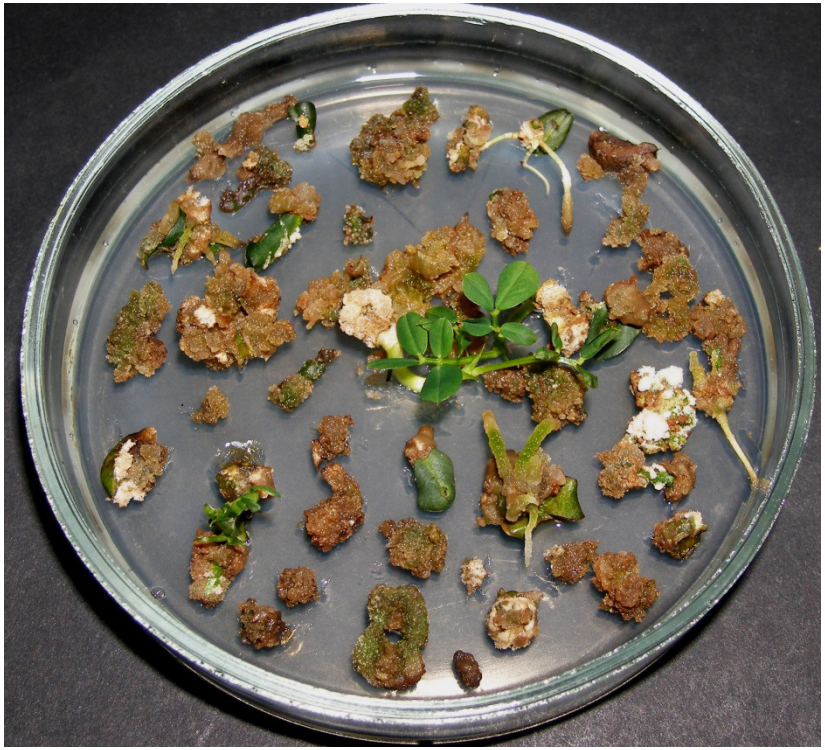
ABSTRACT: Betacyanins and betaxanthins were characterized and determined in an intensely pigmented red-colored callus culture of *Celosia cristata* L. (Amaranthaceae). A new malonyl derivative, 6′-*O*-malonyl-amaranthin (celoscristatin) was isolated and identified by spectroscopic and mass spectrometric techniques. Its stereoisomer, 4′-*O*-malonyl-amaranthin (celoscristatin acyl-migrated), as well as its 15*R* diastereomer were also detected in the callus as a result of the malonyl group migration in



In collaboration with D.Sci, PhD Sławomir Wybraniec
(Cracow University of Technology, Poland)

PLANT GENETIC ENGINEERING

Plant genetic transformation of *Fabaceae* family species



TRANSGENIC *ARACHIS HYPOGAEA* L. PLANTS RESISTANT TO HERBICIDE

Obtaining of transgenic wheat plants using biolistic transformation methods

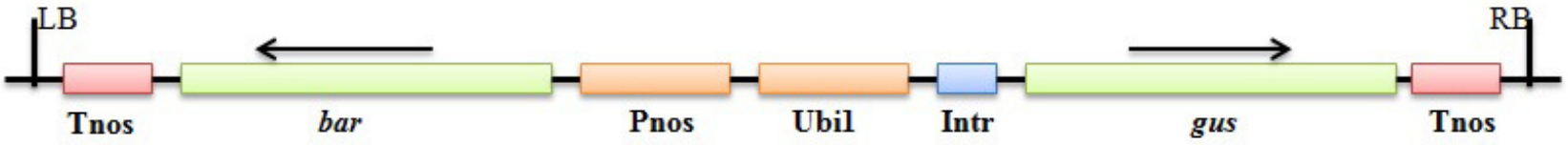
Histochemical staining of GUS activity of weat plants transformed with vector AH025:

GUS activity was detected in flowers and seeds of plants that were transferred to soil in greenhouse

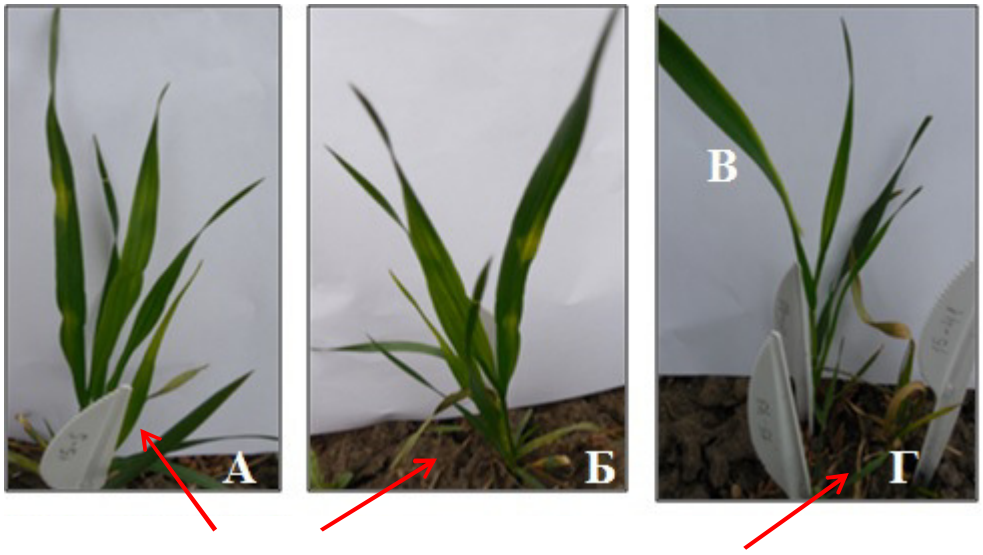
PCR analysis of DNA isolated from wheat plants using primers for *bar* gene:

K- — negative control with DNA from nontransformed plant;
K+—positive control, plasmid pICH025 DNA;
1–5 —DNA from different plants resistant to phosphinotricin;
M—molecular weight marker 1 kb Plus DNA Ladder (Gibco BRL).

Agrobacterium-mediated in Vitro & in Planta Transformation of *Triticim aestivum*



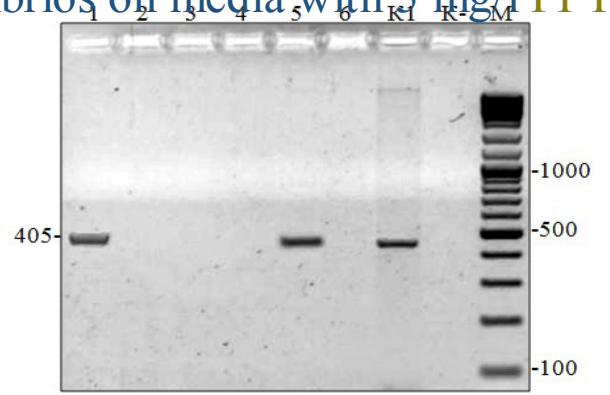
T-DNA with glufosinate-resistant gene (*bar*) of plant expression vector pCB203 in *A. tumefaciens* strain GV3101



Transgenic (Resistant) *Wild-Type (Susceptible)*

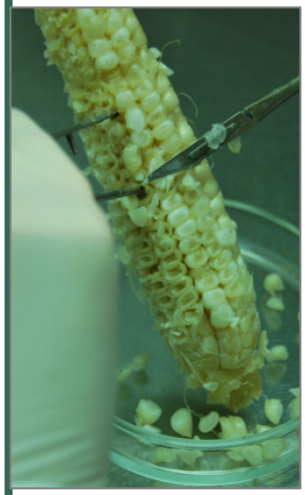
Transformed plants of local cultivar Podolianka treated with herbicide Basta® (1.5 mg/ml)

Regeneration of calli from immature embrios on media with 5 mg/l PPT



Reverse transcription-PCR analysis of transgene *bar* for selected transgenic lines

Biolistic & *Agrobacterium*-mediated Transformation of *Zea mays*



Local genotypes

♀PLS61×♂ДК633/266

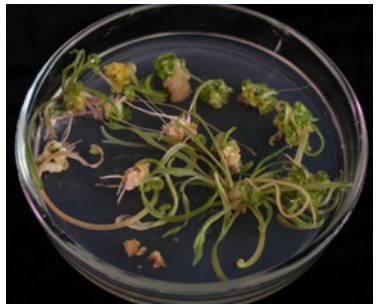
ДК267×PLS61

♀ДК232×♂ДК959

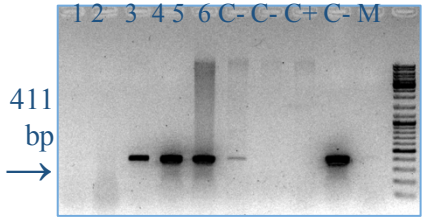
PLS61

Immature embryo

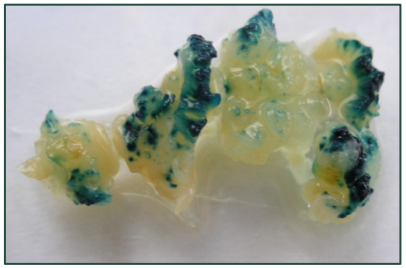
Type I callus



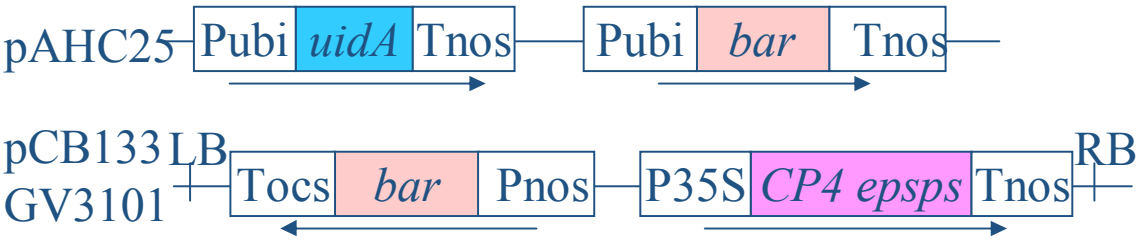
Regeneration



PCR analysis for *bar*

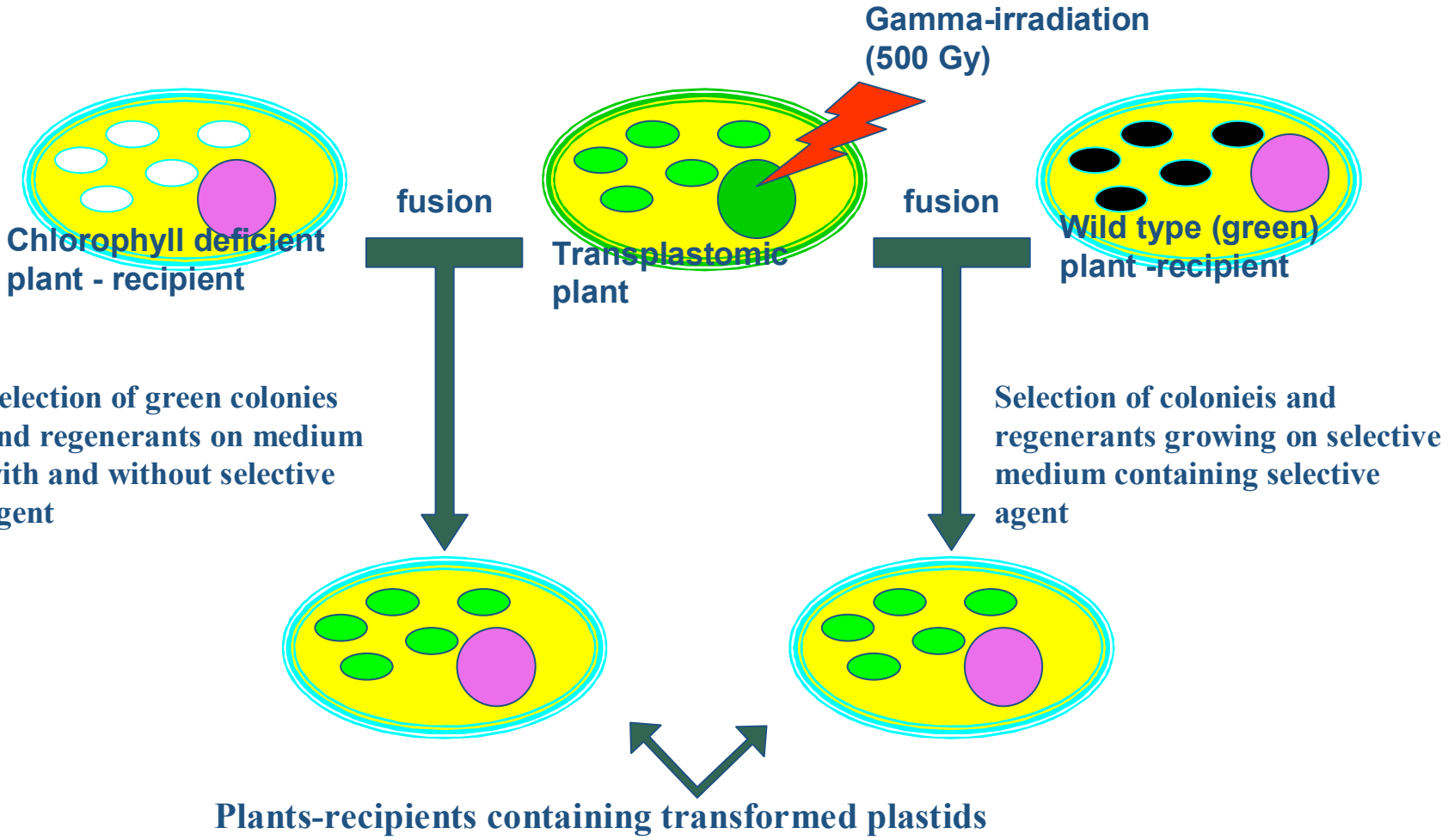


GUS staining

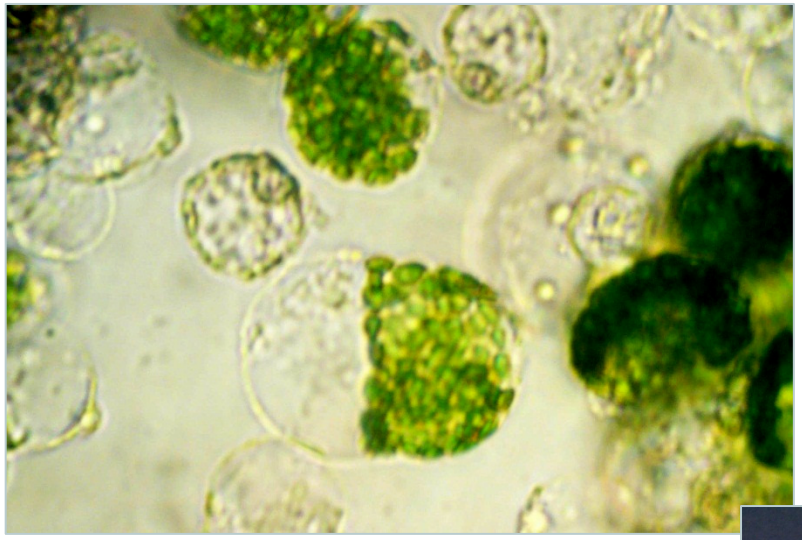


T_2 plants in the greenhouse resistant to herbicide Basta®

Chloroplast transformation «Clipboard» approach

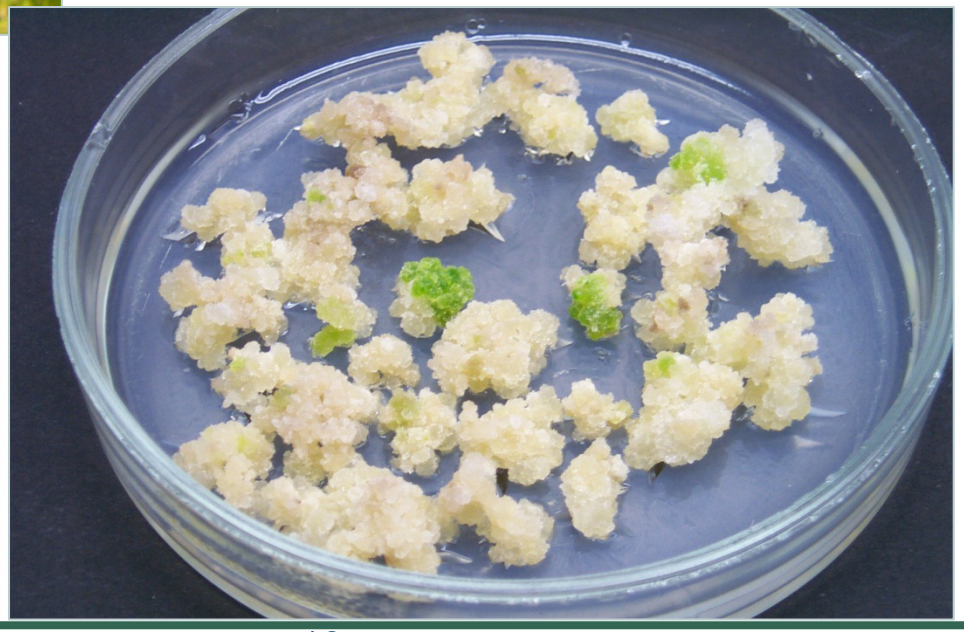


CYBRID DEVELOPMENT

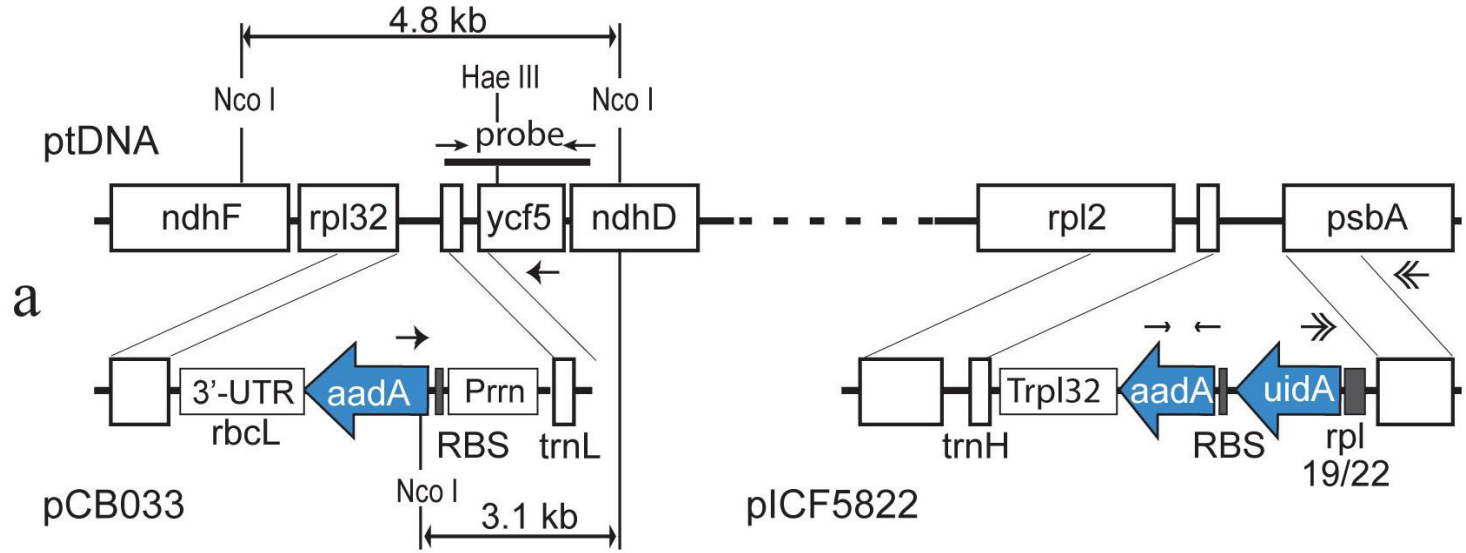


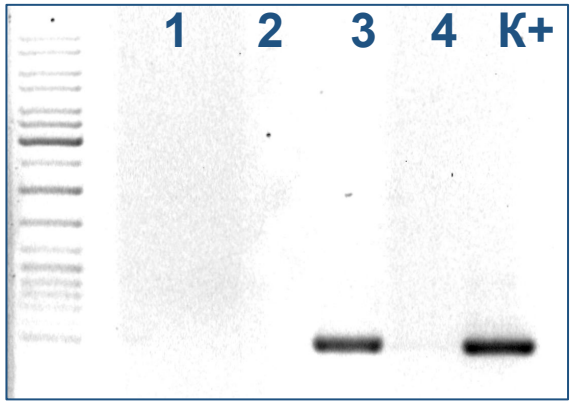
PROTOPLAST FUSION BETWEEN CHLOROPHYLL DEFICIENT RECIPIENT AND IRRADIATED 500GY CHLROPLAST

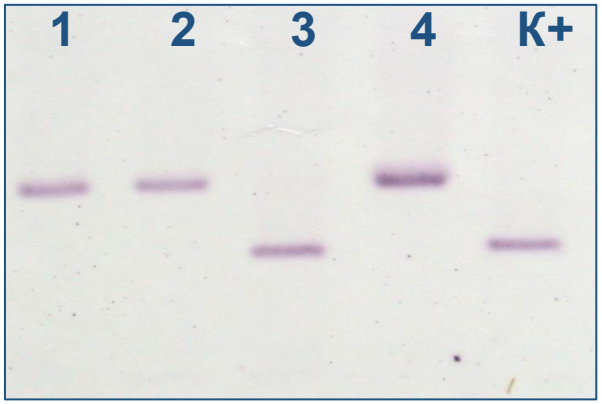
CYBRID SELECTION



PCR AND SOTHERN BLOT ANALYSIS OF TRANSPLASTOMIC *N. TABACUM (ATROPA BELLADONNA)* PLANTS







1-4 - Spm-resitant lines;
K+ - positive control

Somatic hybrids between *Orychophragmus violaceus* (L.) O.E. Schulz and *Lesquerella fendleri* (Gray) Wats.

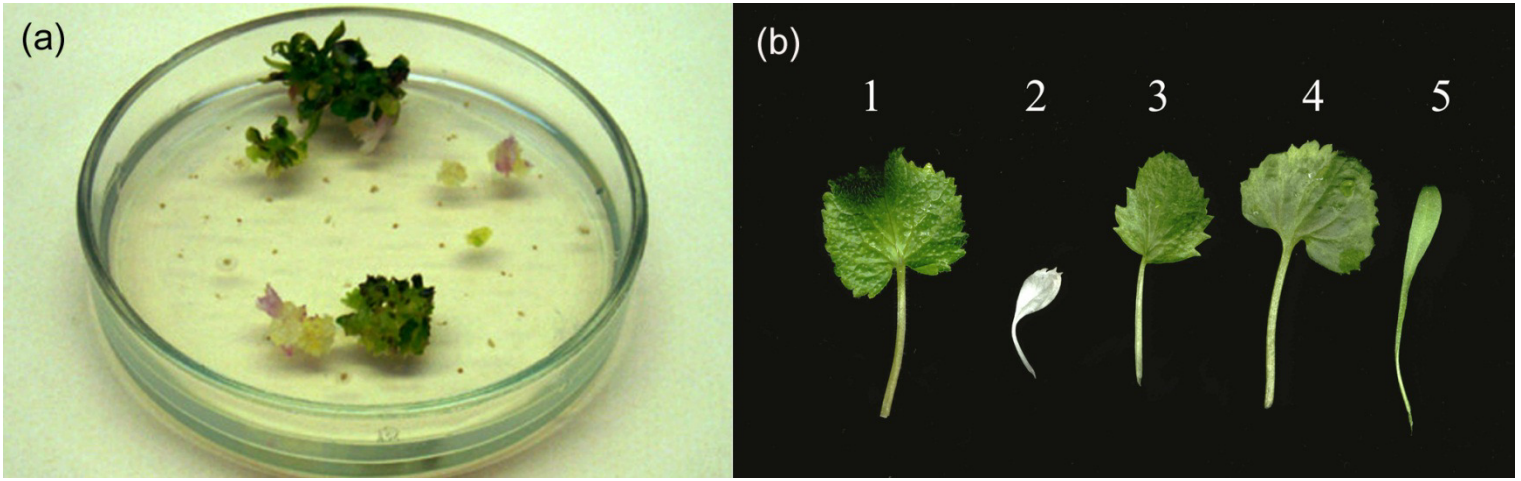
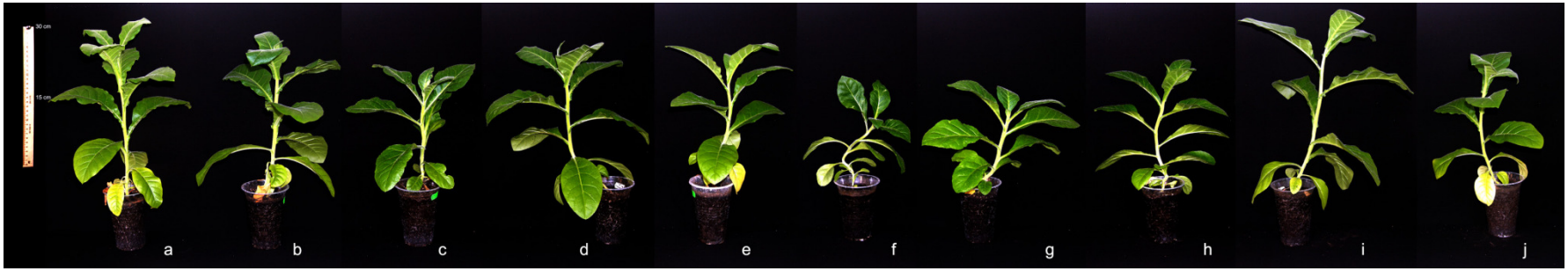


Fig. a Regeneration of hybrids from colonies obtained after protoplast fusion on the streptomycin-spectinomycin free medium. **b** Leaf morphology of *O.violaceus* wild type (1), albino *O.violaceus* (2) hybrid plants (3 - 4), *L.fendleri* (5).

Ovcharenko et al., PCTOC, 2011 (105),21-27

Comparison of effectiveness of 5′-regulatory sequences in transplastomic tobacco chloroplasts

Development of 9 transplastomic *N. tabacum plants with* β-glucoronidase gene under control of different regulatory sequences

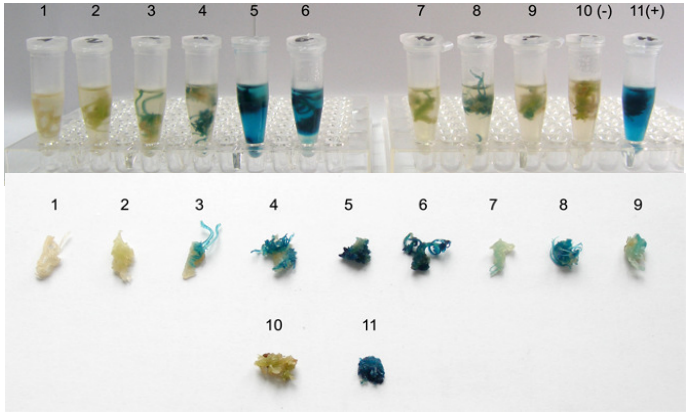


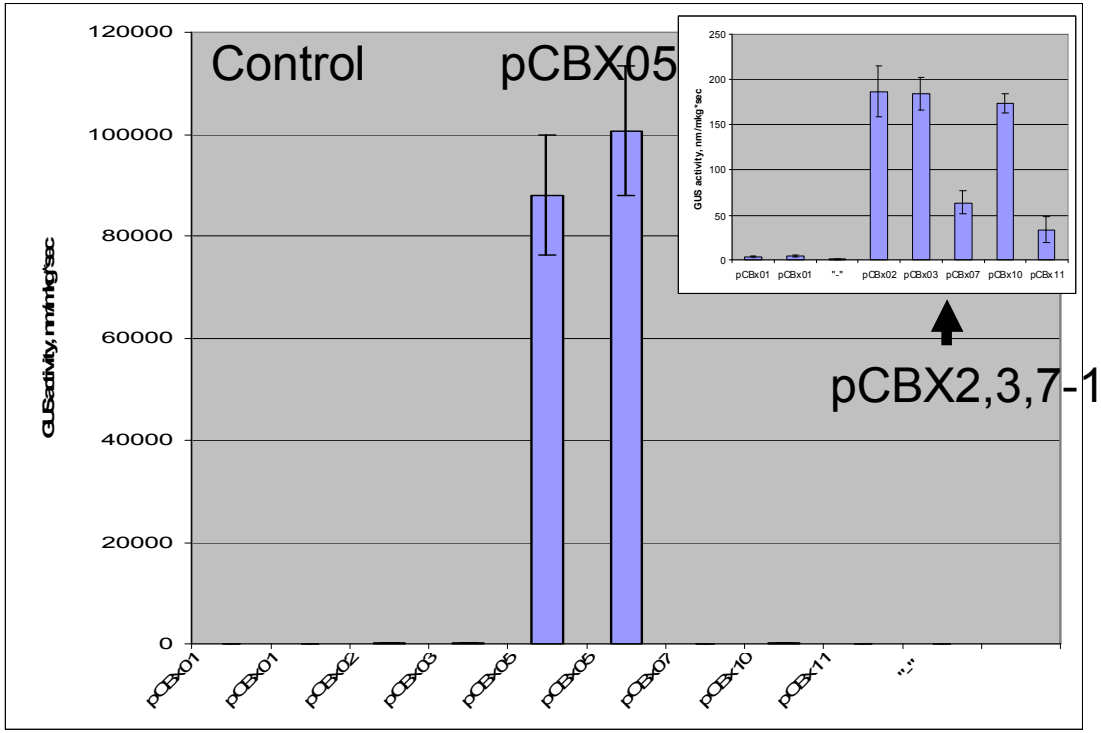
Sourse of sequence:

pCBX02 - *Nicotiana tabacum rbcL*

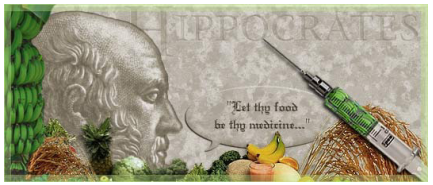
pCBX03 - *Phaseolus vulgaris rbcL*

pCBX05 - *Medicago truncatula*

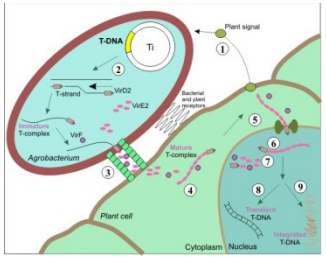


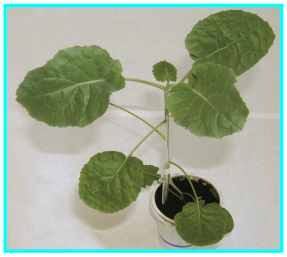


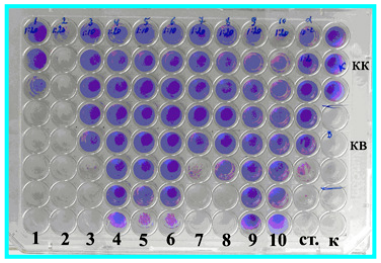
PLANTS AS A SOURCE OF “EDIBLE” VACCINES AND RECOMBINANT PROTEINS

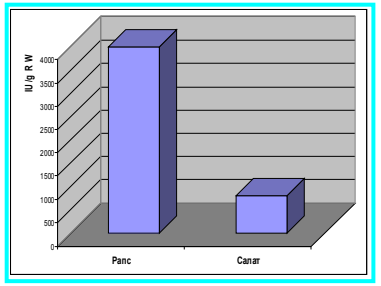


Gene cloning → plant transformation → recombinant protein accumulation → product

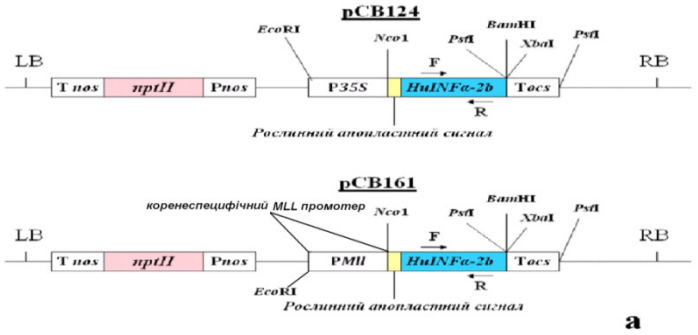




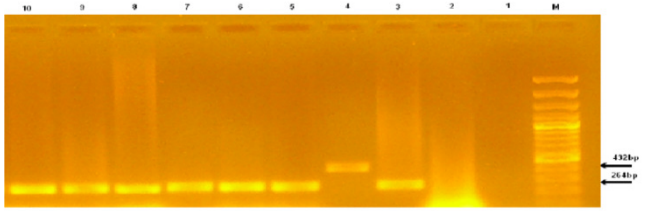




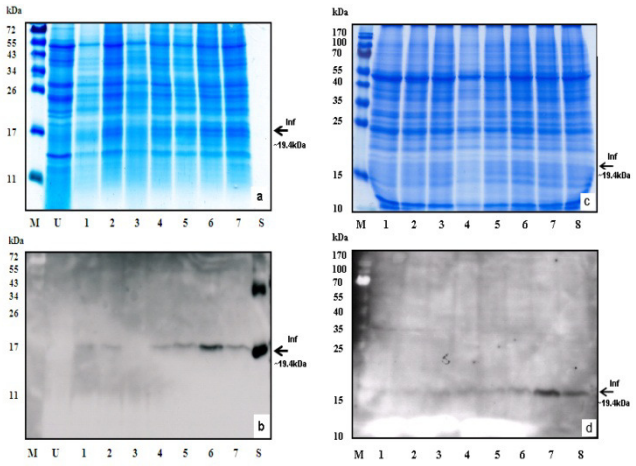
High-level expression of human interferon alpha-2b up to 50,000 IU/g in transgenic carrot

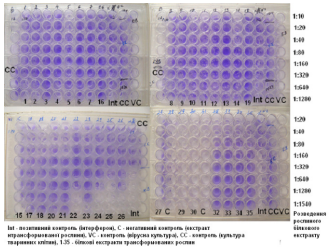


а



б



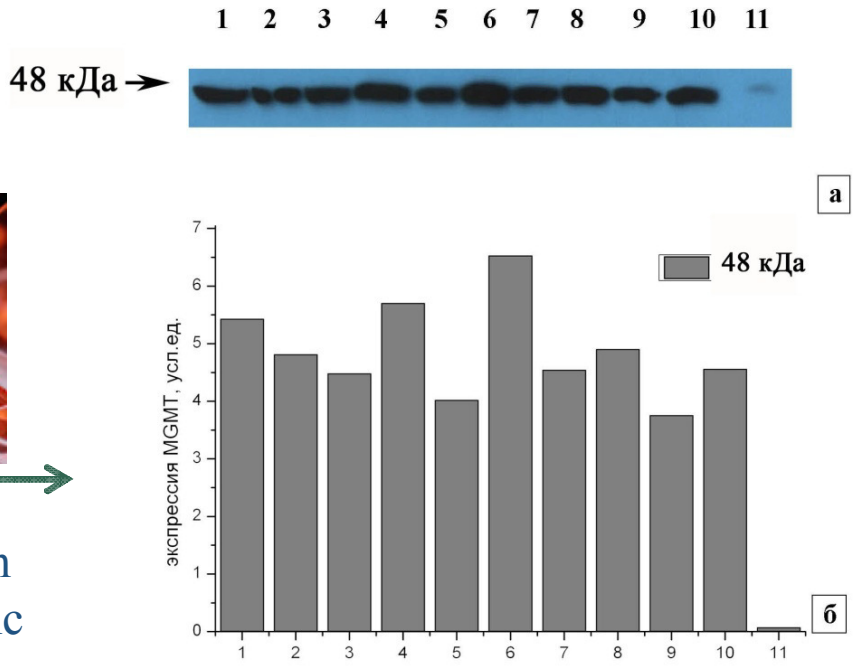


	Interferon activity MO/gm
Young leaves	Up to **50,7×10³**
Taproots	Up to **16,5×10³**
Callus	Up to **1,8·10³**
Suspension culture	Up to **3,5·10³**
“Hairy roots”	Up to **12,08·10³**

Influence of extracts of transgenic carrot plants overexpressing the interferon *α2b* gene on the expression of the MGMT gene in human cells *in vitro*

Transgenic carrot plants expressing human alfa interferon

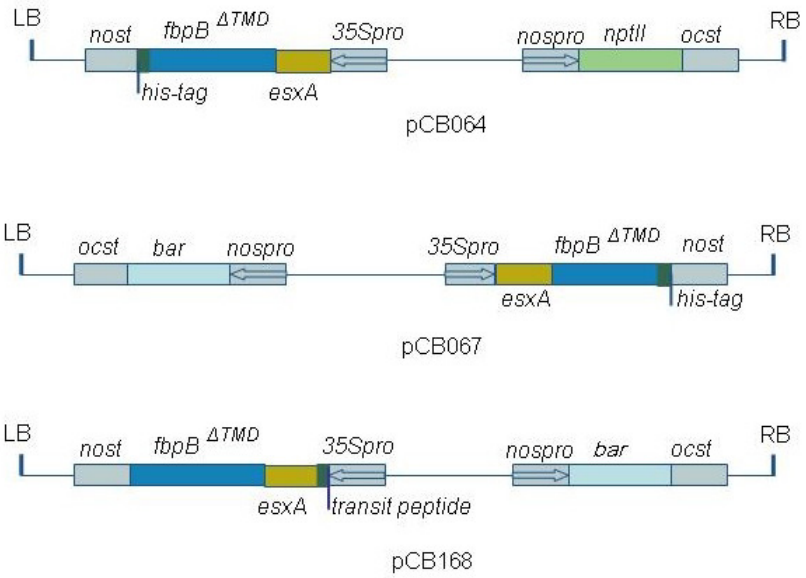
Cell treatment with extracts of transgenic plants overexpressing the interferon alfa

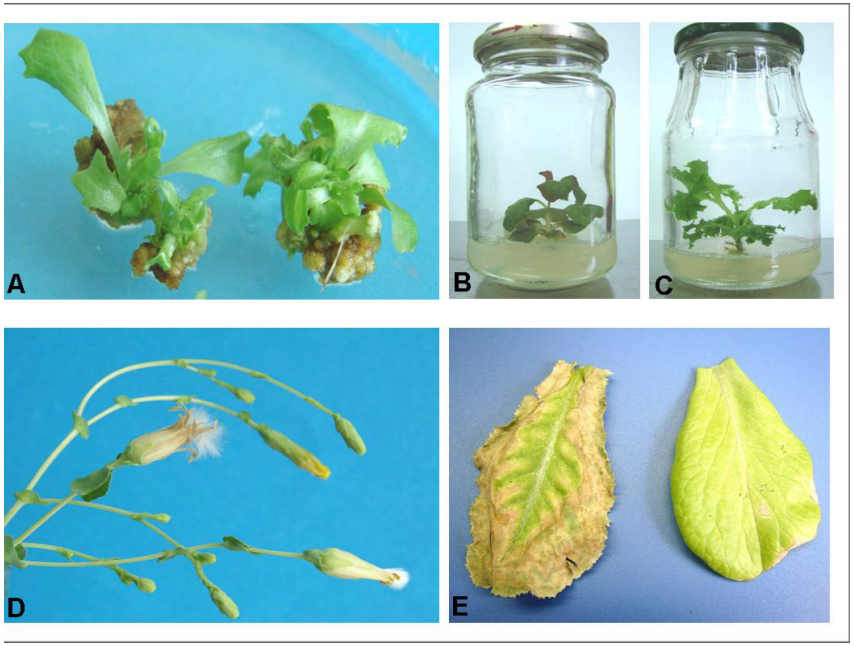


Western blot analysis of the influence of different drugs , containg IFN-α2b on gene MGMT in human cell 4BL

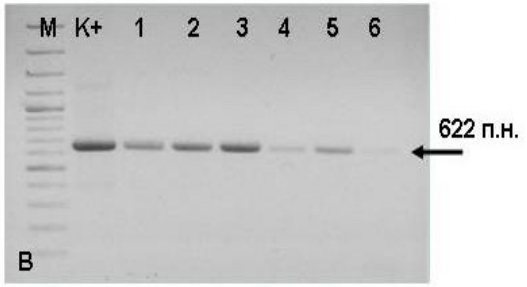
1-control, 2-IFN-α2b (2 IU/ml), 3-IFN-α2b (20 IU/ml), 4-IFN-α2b (200 IU/ml), 5-IFN-α2b (2000 IU/ml) 6-Laferon (2 IU/ml), 7-Laferon (20 IU/ml), 8-Laferon (200 IU/ml), 9-Laferon (2000 IU/ml), 10-extract of transgenic plants with "empty" vector, 11-extract of transgenic plants containing the gene for IFN-α2b (600 IU/ml).

Obtaining of lettuce transgenic plants that contains genes of fusion protein ESAT-6-Ag85b of *Mycobacterium tuberculosis*

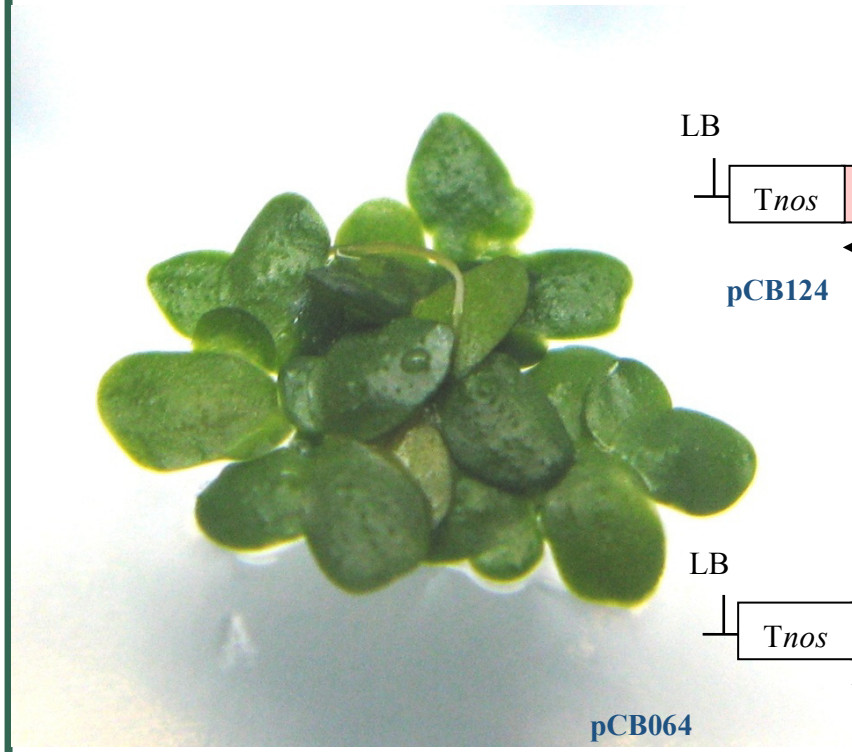




The transgenic plants of lettuce (*Lactuca sativa*) cv. Avstraliyskyy, Odesskiy kucheryaviy, Lolo rossa and Grand rapids have been obtained by *Agrobacterium*-mediated transformation with vectors pCB067, pCB064 and pCB168. These vectors contain gene of fusion protein ESAT-6-Ag85b *Mycobacterium tuberculosis*. Also we have been obtaining the transgenic lettuce plants containing the thaumatin II gene from *Thaumatococcus daniellii* and transgenic lettuce plants producing physiologically active human interferon alpha-2b



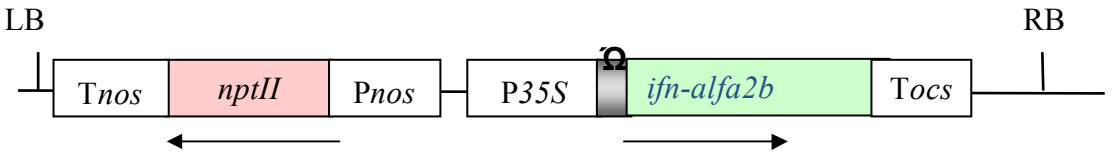
Genetic transformation of *Lemna minor*



Lemna minor

Agrobacterium tumefaciens

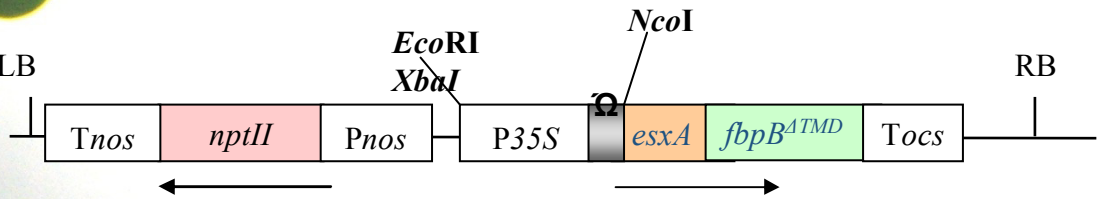
Ifn-α2b



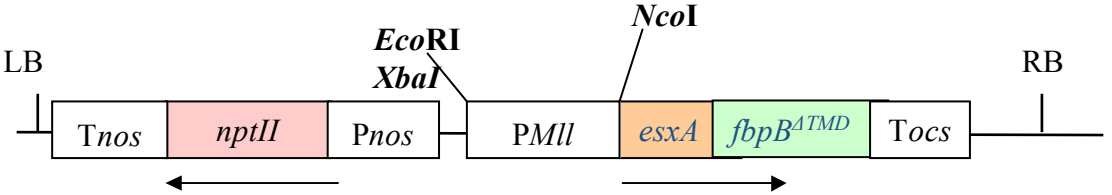
pCB124

Agrobacterium rhizogenes
Agrobacterium tumefaciens

ESAT6 та Ag 85B



pCB064

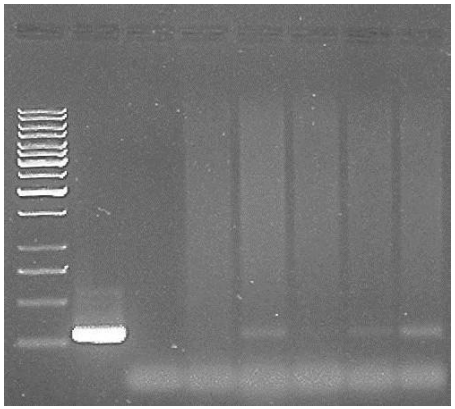


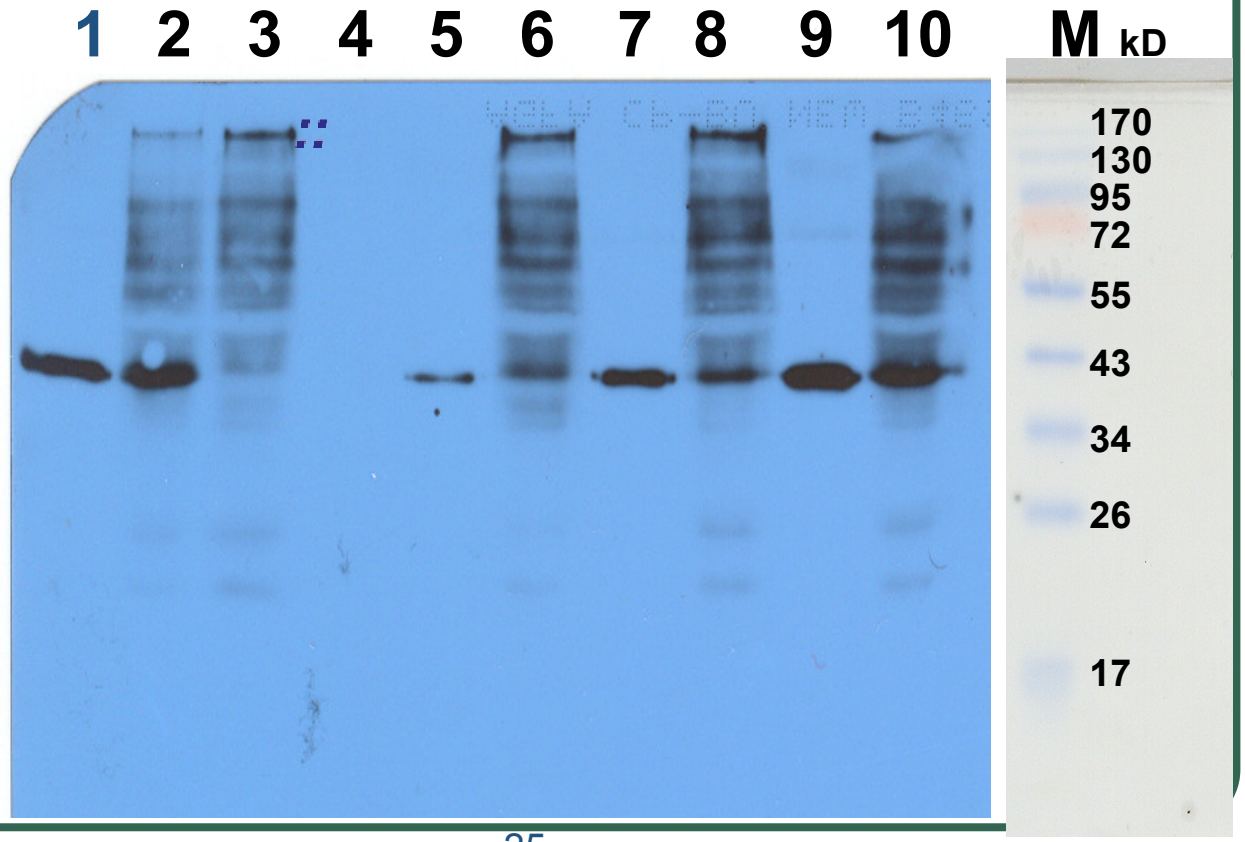
pCB158

Genetic Transformation of Alfalfa Medicago sativa l. with Gene esxafbpb^{dtmd} Coding Mycobacterium tuberculosis Ag85b::ESAT6 fused protein

Western blot analysis. Ag85b::ESAT6 fused protein presents ca. 0.01% TSP

PCR analysis of transgenic alfalfa





Biological activity of transgenic plants containing *Mycobacterium tuberculosis* antigenes

Transgeic carrot and alfalfa

Feeding of Guinea pigs

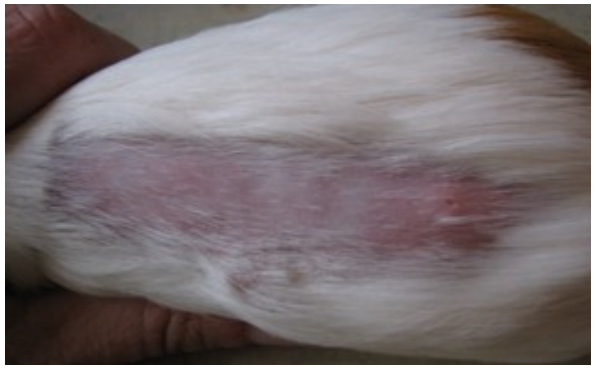
Total dose was ca. 63 mkg* of recombinant protein *per one animal

Tuberculin injections have been done one month after feeding

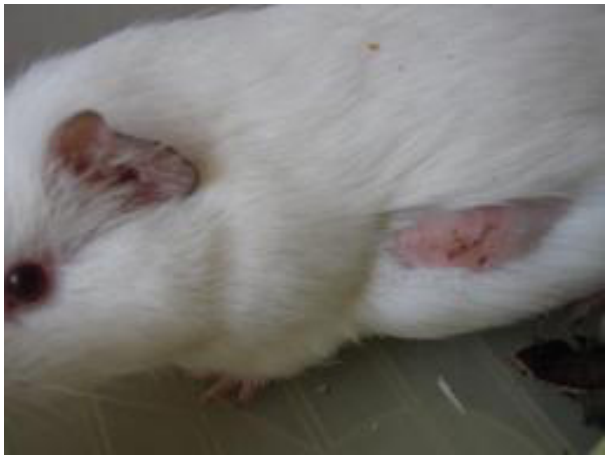
Biological activity of transgenic plants containing *Mycobacterium tuberculosis* antigenes

	First day after injection		2nd day		3rd day		4th day		5th day		6th day	
Control (BCG, 3mg)	1	2	1	2	1	2	1	2	1	2	1	2
	+ +	+	++++	+	+++	-	++	-	++	-	++	-
Experimental animals	+	-	++	-	+++	-	++++	-	++	-	++	-
	-	-	+	-	++	-	+++	-	++	-	++	-
1 – tuberculin												
2 – tuberculin diluted 10 times												

Biological activity of transgenic plants containing antigenes *Mycobacterium tuberculosis*

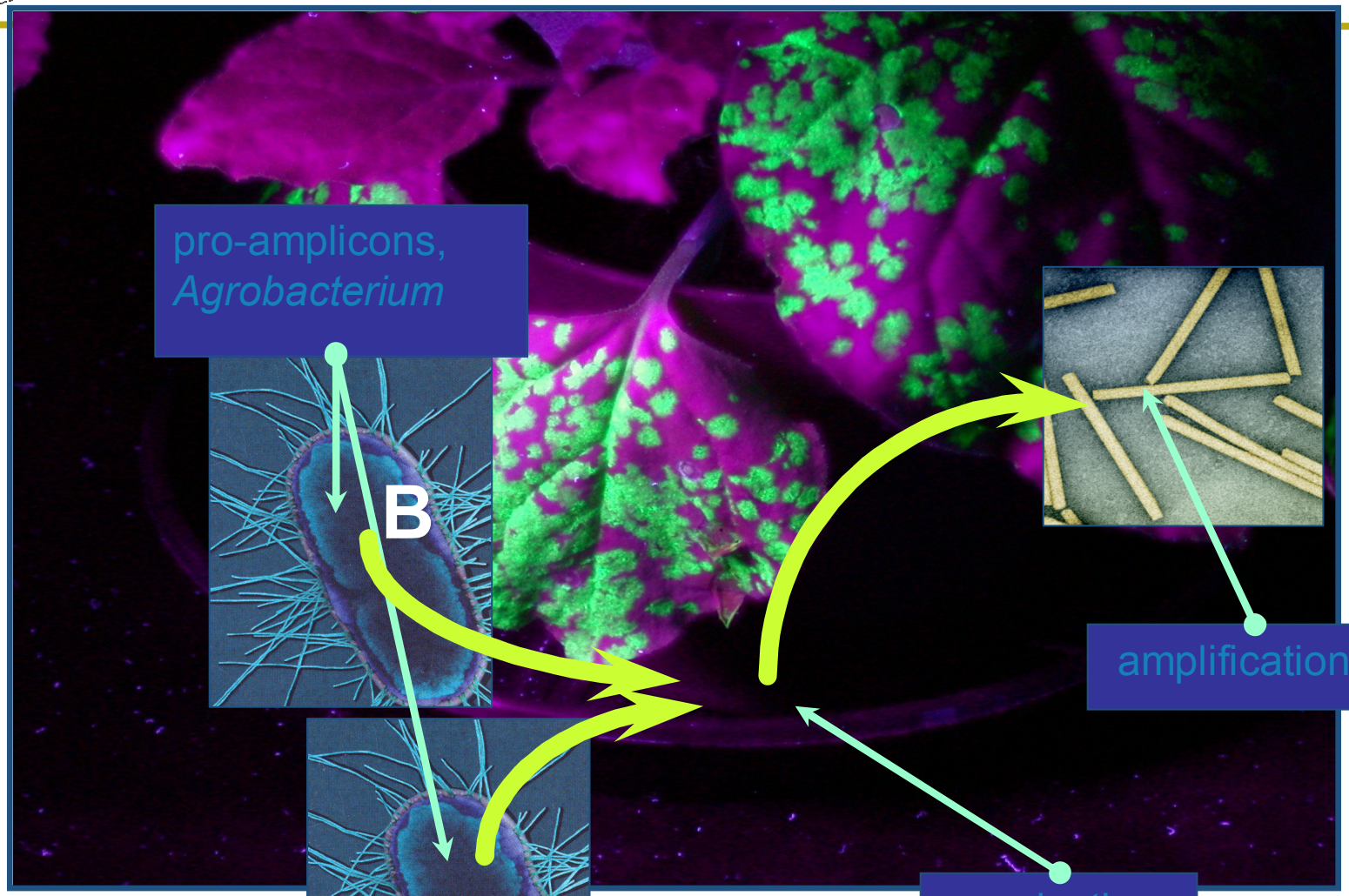


Control



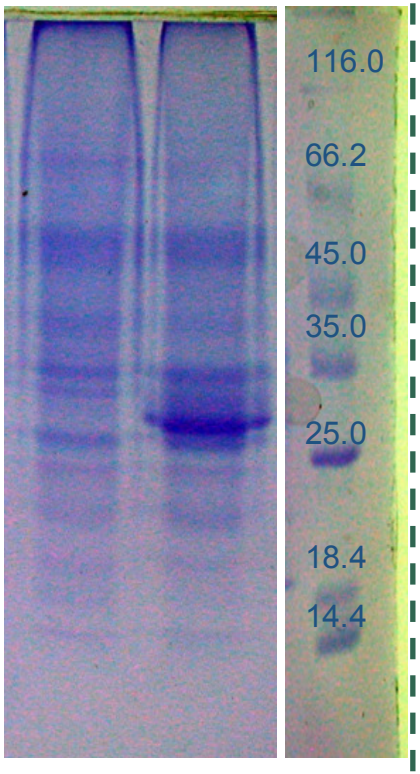
After animal feeding with transgenic plants containing *M. tuberculosis* antigenes

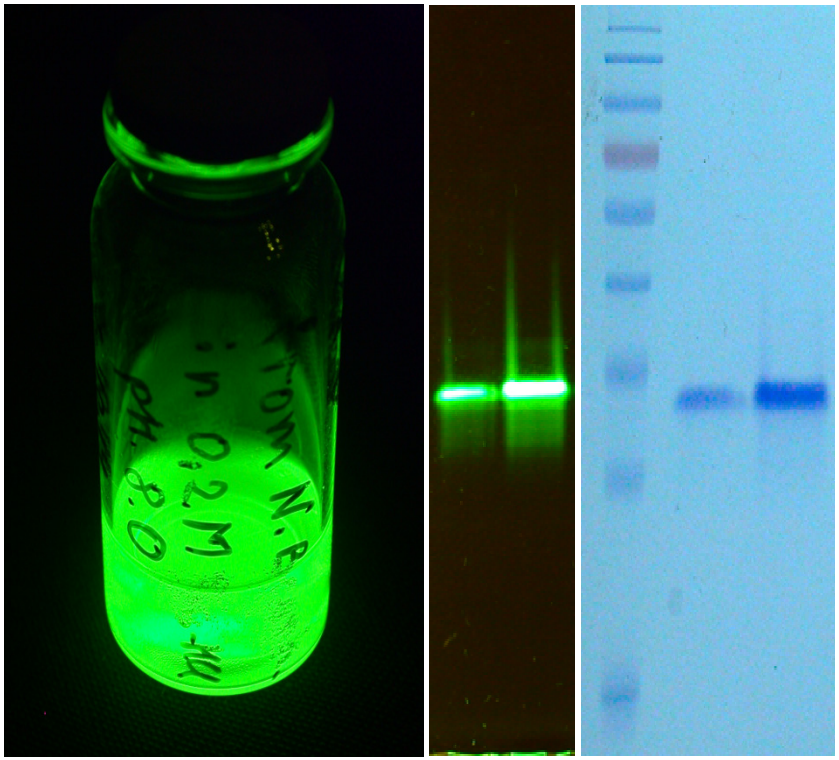
TRANSIENT *AGROBACTERIUM*-MEDIATED GENE EXPRESSION IN *NICOTIANA BENTHAMIANA* PLANTS



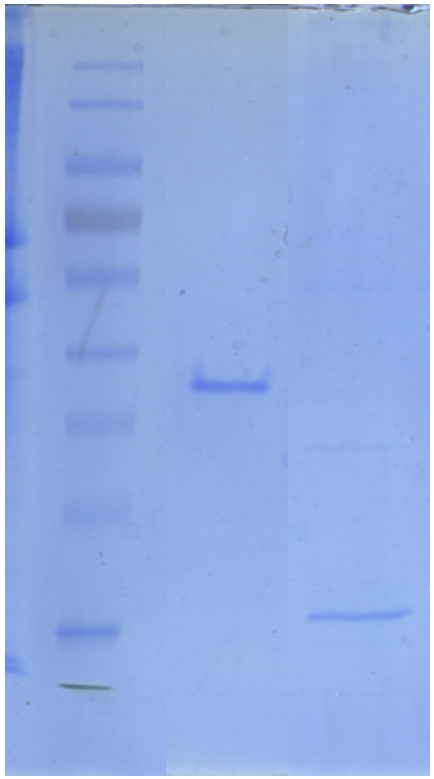
GFP PRODUCTION IN *NICOTIANA BENTHAMIANA* PLANTS

Transient expression → Plant protein extraction → Chromatographic purification





PRODUCTION OF PURIFIED RECOMBINANT INTERFERONE *A2B* AFTER TRANSIENT EXPRESSION INTO NICOTIANA BENTHAMIANA PLANTS



GFP

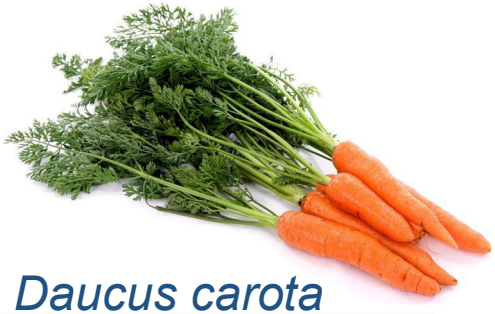
Interferone

0,1±0,02 mg per **100**g fresh plant weight

Purification **>95**

Activity 4 x 10^7 IU/mg

TRANSIENT EXPRESSION IN EDIBLE PLANTS



Daucus carota

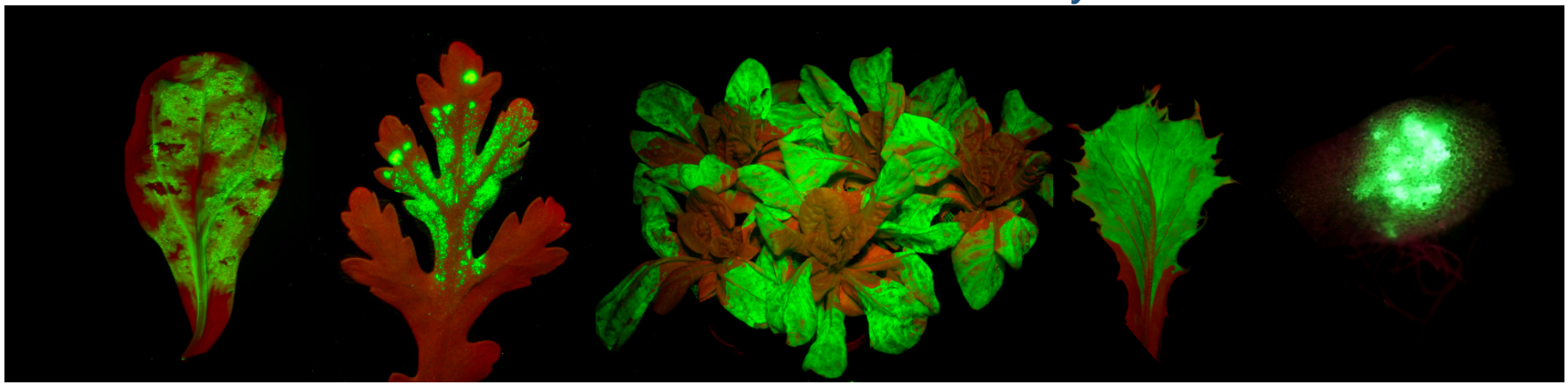
Lactuca sativa

Beta vulgaris

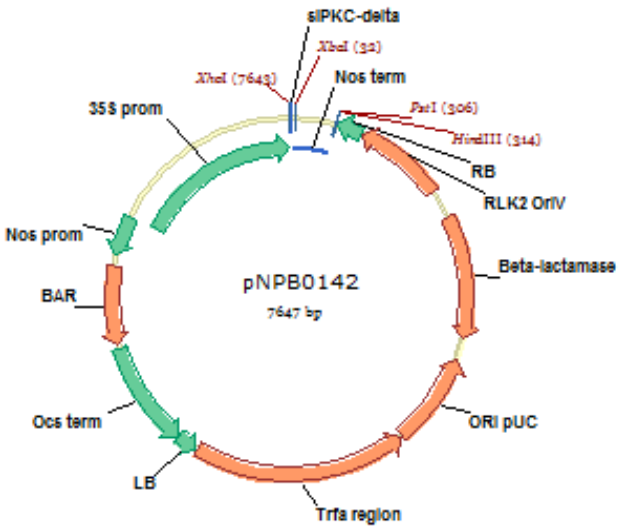
Valirianella locusta

GFP

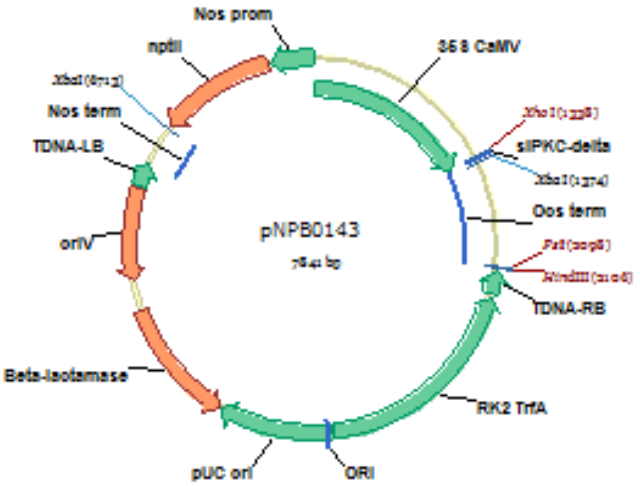
Chrysanthemum coronarium



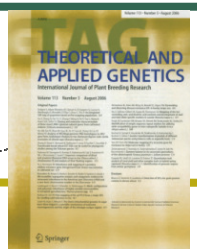
Establishment of potentionaly antihypertensive lettuce plant accumulated micro interfering RNA (miRNA) homological with human proteinkinase C gene



Agrobacterial transformation



Lactuca sativa



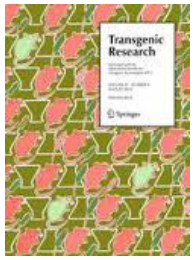
Kuchuk N., Sytnyk K., Vasylenko M., Shakhovsky A., Komarnytsky I.,Kushnir S. Gleba Y. (2006) Genetic transformation of plastids of different Solanaceae species using tobacco cells as organelle hosts. *Theor. Appl. Genet.* 113: 519-527.

Sheludko Y. V., Sindarovska Y. R., Gerasymenko I. M., Bannikova M. A., Kuchuk N. V. (2007) Comparison of several *Nicotiana* species as hosts for high-scale *Agrobacterium*-mediated transient expression. *Biotechnol. Bioeng.* 96, 608-614

Lystvan K., Belokurova V., Sheludko Y., Ingham J.L., Prykhodko V., Kischenko O., Paton E., Kuchuk M. (2010) Production of bakuchiol by in vitro systems of *Psoralea drupacea* Bge. *Plant Cell Tissue Organ Cult.*101, 99-103.

Ovcharenko O., Momot V,, Cherep N., Sheludko Y., Komarnitsky I.,Rudas V. Kuchuk M. (2011)T ransfer of transformed *Lesquerella fendleri* (Gray) Wats. chloroplasts into *Orychophragmus violaceus* (L.) O.E. Schulz by protoplast fusion.*Plant Cell Tissue Organ Cult.*105, 21-27.

Luchakivskaya Y, Kishchenko O., Gerasymenko I.,Olevinskaya Z.,Simonenko Y., Spivak M., Kuchuk M. (2011) High-level expression of human interferon alpha-2b in transgenic carrot (Daucus carota L.) plants. *Plant Cell Rep. 3*0, 407-415.



Gerasimenko M, Sheludko V.V., Klebanoich A. A., Rudas V.A., Shakhovsky A.M., Klein T.M., Kuchuk N.V. (2017) Comparison of effectiveness of 5′-regulatory sequences in transplastomic tobacco chloroplasts. *Transgenic Res.* 26, 65-75.

Institute and "Cytology and Genetics" journal in social nets

- "And he gave it for his opinion, that whoever could make two ears of corn or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind, and do more essential service to his country than the whole race of politicians put together."

  Gulliver's Travels,'A Voyage to Brobdingnag' ch.7 (1726) Jonathan Swift. Anglo-Irish poet and satirist

Acknowledgement

To all colleges and co-workers from Department of Genetic Engineering and Department of Molecular Genetics
Institute of Cell Biology and Genetic Engineering NASU

Thank you !

www.icbge.org.ua

ІКБГІ НАН України