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OBTAINING OF TRANSGENIC BROCCOLI PLANTS WITH ALPHA-2B LEUKOCYTE INTERFERON GENE

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The rapid development of genetic engineering has created opportunities for the production of recombinant proteins for medical purposes. Recombinant DNA technologies solve the problem of shortage of animal raw materials and make it possible to obtain protein preparations (vaccines, antibodies, enzymes and cytokines) using bacterial (mainly *E. coli*) or mammalian (Chinese hamster ovary (CHO) cell lines. However, plants are considered to be a promising expression system for the production of pharmaceutical proteins. Plants have a number of advantages over the other expression systems, one of which is that the use of plants is more environmentally friendly. The usage of plants makes reducing of energy consumption possible, because growing of plants does not require bioreactors, which are used for cultivation of bacterial or animal cells. Recombinant proteins can be stored in plants for a long time, which enables their transportation without refrigerators and greatly simplifies their delivery and storage. In addition, raw-edible transgenic plants may be used as “edible vaccines” and the expensive purification of the product (protein) appears unnecessary, that results in low prime cost of the medicine. The edible vaccines can also stimulate mucosal immunity that can prevent viral diseases. This way, the necessity to use plastic medical syringes can be avoided and the environmental pollution by plastic can be reduced. The use of plant expression systems allows to avoid the risk of contamination of the product with animal viruses and prions or bacterial toxins. Moreover, the medicine with no animal reagents can be used by people with some allergic reactions, vegetarians or some religious communities which do not use the products of animal origin.

Interferons are known for their antiviral, antiproliferative and immunomodulating activity. Interferons are the group of signal proteins that are synthesized by the human body in response to viral or bacterial infections. These proteins activate the mechanisms of blocking viral transcription, viral RNA degradation, moreover, they inhibit the translation in tumor cells and cause apoptosis. Also, the interferons are known for their immunomodulating activity (as they activate T-lymphocytes, NK cells and macrophages). Interferon alpha-2b is the leukocyte protein widely used in medicine for curing viral infections and several types of cancer. As an antitumor protein it is effective in treatment of chronic myeloid leukemia (CML) [1], melanoma [2], liver cancer, Kaposi sarcoma [3] and other. As an antiviral medicine it is effective against human immunodeficiency virus (HIV) [4], SARS-CoV2 [5], hepatitis B, C, D and influenza virus.

The main idea of this study was to obtain transgenic broccoli plants able to express the human interferon alpha 2-b gene. For our study we chose broccoli - an important plant of *Brassicaceae* family. These plants are able to accumulate such minerals and vitamins as C, K and calcium. Also, the young shoots of broccoli plants contain high levels of glucoraphanin that is converted into potential antioxidant substance called sulforaphane during processes of digestion. Sulforaphane is an effective antioxidant known for its antitumor activity as it may reduce the risk of pancreatic cancer, ovarian cancer [6], breast cancer etc. Sulforaphane can also reduce the number of *Helicobacter pylori* colonies, which correlates with the stomach cancer prevention [7]. Therefore, we consider the combination of interferon and sulforaphane may improve the antitumor effect of the obtained transgenic plants.

In our work, we obtained the transgenic plants *via Agrobacterium*-mediated genetic transformation.[8] The experiment on genetic transformation was conducted at the Institute of Cell Biology and Genetic Engineering. For transformation we used *Agrobacterium tumefaciens* strain GV3101 that carries the genetic vector carrying the sequence coding for interferon driven by 35S promoter of cauliflower mosaic virus (CaMV) and phosphinothricinacetyltransferase (*bar*) genes (fig.1.).



Figure. Scheme of T-DNA of the plasmid vector with interferon gene:

LB and RB - left and right boundary sequences of T-DNA; 35S - 35S promoter of CaMV; *infa2b* - human interferon alpha 2b gene; *bar* - selective phosphinothricinacetyltransferase gene.

Broccoli seeds of Valtam, Battavia and Romanesco varieties were surface sterilized by 70% ethanol for 1 min, followed by 15 minutes in 40% bleach solution (2% sodium hypochlorite) and then rinsed several times in sterile distilled water. The seeds were germinated on MS medium and then grown at + 24°C and a 16-hour photoperiod.

We used 1 cm long hypocotyl explants of 10-day aseptic seedlings for transformation. Before co-cultivation with *A. tumefaciens*, the explants were pre-cultivated on MS medium with 2 mg / l of BA for 4-7 days. The bacterial suspension was cultured overnight in liquid LB medium at +28°C on rotary shaker (200 rpm) with carbinicillin 100 mg / l, rifampicin 100 mg / l and gentamicin 25 mg / l. The night bacterial culture was precipitated by centrifugation (4500 rpm) and resuspended in liquid MS medium. The broccoli explants were co-cultivated for two-hours with bacterial suspension culture, after that the explants were incubated on wet sterile blotting paper for 48 h in dark. After co-cultivation the explants were transferred on MS medium containing 500 mg / l ceftriaxone for bacterium elimination, 2 mg / l 6-benzyladenine (BA) and 0.05 mg / l naphthaleneacetic acid (NAA) for plant regeneration and 5 mg / l phosphinothricin as the selective agent. The explants were incubated at + 24 °C and a 16-hour photoperiod and transferred to fresh medium every two weeks. In two weeks after transformation we observed the formation of callus on the inoculated explants. After 7-8 weeks of callus clone cultivation on the selective regenerative medium, the green broccoli shoots 1–2 cm long were formed on the explants. The most active callus formation and regeneration on the medium with selective agent was observed for the explants of Romanesco broccoli variety.

As a result of our study, we have obtained phosphinothricin-resistant broccoli plants of the Romanesco variety. These plants are potentially transgenic and may contain the interferon gene in their genome. When the plants are characterized with sufficient biomass, we are going to prove the presence of interferon gene in this plant genome by PCR analysis. Moreover, we are going to analyze the antiviral and antiproliferative activity of the obtained plant extracts.

Conclusions:

1. The broccoli plants of three varieties Valtam, Battavia and Romanesco were introduced into *in vitro* culture by surface sterilization of seeds.
2. The hypocotyl explants of Romanesco variety plants demonstrated the highest regenerative potential.
3. The phosphinothricin-resistant broccoli plants of Romanesco variety were obtained via genetic transformation and these plants potentially contain the interferon gene in their genome.

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