UDK 575.222.7:581.1 doi: 10.15407/biotech8.02.026

# USING OF *Agrobacterium*-MEDIATED TRANSFORMATION FOR THE BIOTECHNOLOGICAL IMPROVEMENT OF COMPOSITAE PLANTS. II. SYNTHESIS OF BIOACTIVE COMPOUNDS IN TRANSGENIC PLANTS AND «HAIRY» ROOTS

*N. A. MATVIEIEVA*

Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, Kyiv

*E-mail: joyna56@gmail.com*

Received 25.02.2015

The review focused on the data concerning current state in the field of Compositae "hairy" roots and transgenic plants construction using *A. tumefaciens*- and *A. rhizogenes*-mediated transformation to obtain biologically active compounds, including recombinant proteins. The article presents data on the results of genetic transformation of *Cichorium intybus*, *Lactuca sativa*, *Artemisia annua*, *Artemisia vulgaris*, *Calendula officinalis*, *Withania somnifera* and other Compositae plants as well as studies on the artemisinin, flavonoids, polyphenols, fructans and other compounds accumulation in transgenic plants and roots.

The data show that the use of biotechnological approaches for construction of "hairy" roots and transgenic plants with new features are of great interest. The possibility of increase in the accumulation of naturally synthesized bioactive compounds and recombinant proteins production via *A. tumefaciens* and *A. rhizogenes*-mediated transformation have been shown. In vitro cultivation of transgenic plants characterized by high level of bioactive compounds accumulation and synthesis of recombinant proteins makes it possible to obtain guaranteed pure raw material. Using of biotechnological approaches preserved natural populations of plants is particularly important for rare and endangered plant species.

***Key words:*** Compositae, *Agrobacterium tumerfaciens*, *Agrobacterium rhizogenes*, transgenic plants "hairy" roots, biologically active compounds, recombinant proteins.

Genetic engineering is a rapidly developing branch of biotechnological research. The tasks of genetic engineering are diverse. They include researches aimed at fundamental investigations of transferred genes functioning and also an applied research aimed at developing of biotechnology for practical and commercial application. The latter include the development of strategies of biologically active compounds production from plant material using modern biotechnological approaches.

Naturally synthesized compounds and recombinant plant-derived compounds (animal or microbial origin) are among the substances of practical interest. Use of modern biotechnological techniques including genetic engineering methods allow to create medical plants with significantly increased level of accumulation of biologically active substances (BAS), secondary metabolites and stored sugars. Genetic engineering methods application allows constructing so called "edible vaccines" — edible plants which synthesize viral and bacterial recombinant antigens or transgenic plants as a source of another recombinant compounds for medical purposes. Compositae family includes numerous plant species used on medicine. These plants are of special interest for improvement via genetic engineering methods as a source of BAS.

*Increasing of accumulation of biologically active compounds in transgenic plants and "hairy" roots*

Obtaining of plants that can find practical application as a system for BAS synthesis is an ultimate goal of experiments in plant genetic engineering. Up to now numerous laboratories

carry out studies aimed at obtaining of biotechnological plants for producion these compounds in high quantities in comparison with the ones production in nontransgenic plants. The level of accumulation of valuable compounds in plants obtained after transformation of *Agrobacterium tumefaciens* can greatly exceed that the one in the control plants.

Transformation using *A. rhizogenes* is the way for so-called "hairy" root culture obtaining. These roots as well as transgenic plants accumulate BAS quite often in greater amount than the basal nontransformed plant. This increase is a result of foreign genes transfer to the plant genome. Selection of the most productive "hairy" root lines, which are characterized by rapid growth and high BAS accumulation level, their cultivation in bioreactors is a basis of creation of industrial biotechnologies for manufacturing of compounds with medical properties.

*Artemisia* sp. Compositae family plants are of great interest as a source of biologically active compounds. These plants synthesize artemisinin compound with antimalarial properties. En effective antimalarial medicine Coartem (Novartis International AG) based on one of the semi-synthetic derivatives of artemisinin is not produced in Ukraine now. Interest in artemisinin production increased due to high efficiency of this compound in malaria treatment [1, 2] as opposed to earlier and now applied medicines based on quinine. The latter have a number of contraindications, side effects and are not effective against some types of malaria parasites (*Plasmodium falciparum* is resistant to aminohinolons).

*A. rhizogenes* and *A. tumefaciens* are used for wormwood plants transformation in order to construct lines with high artemisinin content. Artemisinin content and essential oils accumulation were studied in *A. annua* transgenic roots and plants using chromatographic and masspectrometry methods. "Hairy" roots growing conditions in bioreactors and possible ways of BAS synthesis increasing (Table 1) were also determined including the optimization of the culture medium composition, the choice of transgenic root lines with high level artemisinin accumulation and transfer in plant genome genes that indirectly increase artemisinin synthesis.

Chen et al. [3] transferred farnesyl diphosphate synthase (FDS) gene in one of the first experiments to obtain *Artemisia annua* transgenic roots and founded difference in growth rate of transgenic root lines and artemisinin accumulation up to 2–3 mg/g roots dry weight. The authors obtained *A. annua* transgenic plants using *A. tumefaciens*-mediated transformation. These plants synthesized and accumulated artemisinin up to 10 mg/g weight that is 2-3 fold higher than the content of the compound in the control plants [4]. In transgenic plants obtained by Han et al. [5] artemisinin accumulated in amount up to 0.9% dry weight, which is 34.4% more than in the control. Banyai et al. [6] also obtained transgenic *Artemisia* plants with 2.5–3.6 fold increased artemisinin accumulation as compared with the control. The results depended on the vector used for the transformation. It should be noted that the researchers used *FPS* gene for the transformation and confirmed thereby regulatory role of farnesyl pyrophosphate synthase in the artemisinin synthesis.

Transfer of isopentyl transferase *ipt* gene has lead to an increase of cytokinins and chlorophyll content in transgenic *A. annua* plants. This phenomenon resulted in increase of artemisinin content in transgenic plants up to 70% [7]. High level artemisinin synthesis also observed after *Artemisia* plants transformation using HMG-CoA reductase and amorpha-4,11-diene synthase genes [8], beta-caryophyllene synthase gene [9], *ADS, CYP71AV1* and *CPR* genes [10], *AaWRYK1* gene [11] *HMGR* and *FPS* genes [12]. Artemisinin synthesis level had any changes in transgenic plants with *fpf1* gene (flowering promoting factor1) from *Arabidopsis thaliana*, but transfer of this gene has led to early flowering [13, 14]. Giri et al. constructed transgenic roots using different strains of agrobacteria and identified differences in the artemisinin amount [15].

Variation in cultivation conditions can also lead to changes in the artemisinin accumulation. Weathers et al. [16] studied the effects of some factors such as the content of auxins, cytokinins, hybberelic and abscisic acid and ethylene content on the artemisinin accumulation in *A. annua* roots and have identified that 2-izopentyladenine addition to the culture medium stimulated artemisinin production. The cultivation of transgenic *A. annua* roots in different types of bioreactors was evaluated and cultivation process parameters were defined [17–20]. *A. dubia, A. annua, A. absinthium, A. indica, A. vulgaris, A. absinthium* transgenic roots also were obtained and the influence of the composition of the culture medium (macro-, microsalts and sucrose concentration) on the growth rate of the roots was studied [21–23].

*Table 1.* **Increasing of secondary metabolites and storage compounds accumulation in plants of Compositae family due to transformation**

| Plant species | Transformation using | The results of transformation | References |
|---|---|---|---|
| *Artemisia annua* | *A. rhizogenes* | Transgenic roots with farnesyl diphosphate synthase gene were characterized by different growth rate and artemisinin content (2–3 mg/g DW) | [3] |
| *Artemisia annua* | *A. tumefaciens* | Transgenic plants synthesized artemisinin up to 10 mg/g (farnesyl diphosphate synthase gene was used for the transformation) | [4] |
| *Artemisia annua* | *A. tumefaciens* | Transgenic plants synthesized artemisinin up to 0.9% DW (farnesyl diphosphate synthase gene was used for the transformation) | [5] |
| *Artemisia annua* | *A. tumefaciens* | Transgenic plants were constructed using farnesyl pyrophosphate synthase gene. These plants accumulated artemisinin in 3.6 fold more amount than the control plants | [6] |
| *Artemisia annua* | *A. tumefaciens* | Transgenic plants carried ipt gene accumulated artemisinin | [7] |
| *Artemisia annua* | *A. tumefaciens* | *A. annua* plants over-expressed HMG-CoA reductase and amorpha-4,11-diene synthase genes. Influence of these genes expression on artemisinin content was studied | [8] |
| *Artemisia annua* | *A. tumefaciens* | Artemisinin biosynthesis enhancement in transgenic *A. annua* plants by downregulation of the beta-caryophyllene synthase gene was studied | [9] |
| *Artemisia annua* | *A. tumefaciens* | Artemisinin biosynthesis in transgenic *A. annua* plants by overexpressing ADS, CYP71AV1 and CPR genes was studied | [10] |
| *Artemisia annua* | *A. tumefaciens* | Overexpression of AaWRYK1 gene increased artemisinin content in *A. annua* plants | [11] |
| *Artemisia annua* | *A. tumefaciens* | Co-overexpression of the HMGR and FPS genes enhanced artemisinin content in *A. annua* plants | [12] |
| *Artemisia annua* | *A. tumefaciens* | The effects of fpf1 and CO genes on *A. annua* flowering time and artemisinin biosynthesis was studied | [13], [14] |
| *Artemisia annua* | *A. rhizogenes* | Influence of different *Agrobacterium rhizogenes* strains on "hairy" root induction and artemisinin production in *A. annua* was studied | [15] |
| *Artemisia annua* | *A. rhizogenes* | The effect of phytohormones on growth and artemisinin production in transgenic *A. annua* roots was defined | [16] |
| *Artemisia annua* | *A. rhizogenes* | Growth of *A. annua* "hairy" roots in bioreactors for BAS production was demonstrated | [17], [18], [19], [20] |
| *Artemisia dubia and Artemisia indica* | *A. rhizogenes* | *A. dubia* and *A. indica* "hairy" roots were inducted and artemisinin accumulation was analyzed | [21] |
| *Artemisia vulgaris* | *A. rhizogenes* | The conditions for *A. rhizogenes*-mediated transformation of *A. vulgaris* were optimized. Essential oils accumulation was studied | [22] |
| *Artemisia absinthium* | *A. rhizogenes* | *A. absinthium* transgenic root culture was initiated | [23] |
| *Lactuca sativa* | *A. tumefaciens* | Increasing of flavonoids and polyphenols content in transgenic plants with CHI gene was detected | [24] |
| *Lactuca sativa* | *A. tumefaciens* | Increase of inulin accumulation occurred in transgenic plants overexpressed asparagine synthetase A gene from *Escherichia coli* | [25] |
| *Cichorium intybus* | *A. tumefaciens* | Inulin was synthesized in transgenic plants harbouring onion fructan:fructan 6G-fructosyltransferase gene | [26] |

| Plant species | Transformation using | The results of transformation | References |
|---|---|---|---|
| *Cichorium intybus* | *A. rhizogenes* | Production of esculin by *C. intybus* "hairy" roots was studied | [27] |
| *Cichorium intybus* | *A. rhizogenes* | Fungal elicitors using enhanced growth and coumarin production in *C. intybus* "hairy" root culture | [28] |
| *Cichorium intybus* | *A. rhizogenes* | Chicory "hairy" roots were cultivated in bioreactors of different configurations | [29] |
| *Cichorium intybus* | *A. rhizogenes* | Fructan accumulation increased in transgenic roots | [30] |
| *Arnica montana* | *A. rhizogenes* | Thymol derivatives synthesis in "hairy" roots of *A. montana* was detected | [31] |
| *Calendula officinalis* | *A. rhizogenes* | Production of oleanolic acid glycosides by *C. officinalis* "hairy" roots was analyzed | [32] |
| *Ocimum basilicum* | *A. rhizogenes* | Rosmarinic acid accumulated in "hairy" root of *O. basilicum* | [33] |
| *Withania somnifera* | *A. rhizogenes* | Withanolide accumulated in transgenic roots | [34], [35] |
| *Tragopogon porrifolius* | *A. rhizogenes* | *T. porrifolius* transgenic root lines differed in fructan accumulation level | [36] |

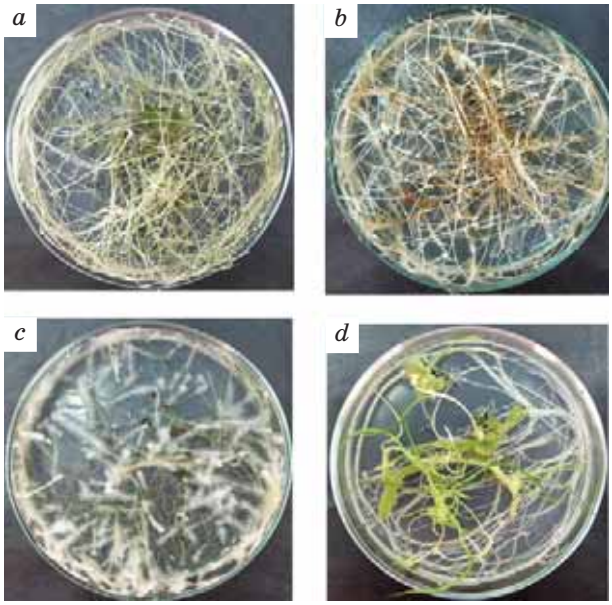
*Cichorium intybus* and *Lactica sativa* edible plant (Compositae) are of interest for genetic transformation experiments. Transgenic lettuce plants obtained via *Agrobacterium tumefaciens*-mediated transformation (chalcone isomerase gene of chinese cabbage) were characterized by 1.4–4.0-fold flavonoids content increase. The results of this study have shown the possibility of construction of lettuce plants with a high content of flavonoids. Authors also determined the role of *CHI* gene in the synthesis of these compounds [24].

Compositae plants are known to synthesize fructans. These compounds exhibit a wide range of biological activity — gepatoprotective, prebiotic activities, they normalize lipid and calcium metabolism. Sobolev et al. [25] noted 30-fold increase of the content of inulin in transgenic lettuce plants with the *Escherichia coli* asparagine synthetase A gene. Transfer of *Allium cepa* 6G-FFT fructan:fructan 6G-fructosyltransferase gene to the chicory plant has led to neoinulin synthesis (Liliaceae storage fructan) in transgenic plants [26]. Transgenic chicory roots enabled synthesis of esculin. Amount of esculin in these roots was 13-fold higher than in the roots of nontransformed plants [27]. Enhancement of growth and coumarin production in "hairy" root cultures of *Cichorium intybus* under the influence of natural fungal elicitors [28] and performance of "hairy" roots cultivation in bioreactors of different configurations [29] were studied. Transgenic *Cichorium intybus* and *Lactuca sativa* "hairy" roots and regenerated plants (Figure) using *A. rhizogenes*-mediated transformation were obtained in our experiments. It was shown that some "hairy" roots lines accumulated fructans in greater amount than the roots of the control plants. Charkor™, Ivin™ and Biolan™ growth regulators using stimulated fructans accumulation in transgenic roots [30].

*A. rhizogenes* bacteria were used to obtain transgenic *Arnica montana* [31], *Calendula officinalis* [32], *Ocimum basilicum* [33], *Withania somnifera* [34, 35], *Tragopogon porrifolius* [36] "hairy" roots (Figure). Transgenic *Calendula officinalis* roots synthesized an oleanolic acid and *Ocimum basilicum* "hairy" roots accumulated rosmarinic acid. The results of these experiments indicate that the approach aimed at the transgenic roots construction and obtaining of compounds with valuable properties are promising and can bring significant economic benefits in case of selection of the most productive transgenic lines.

*Construction of plants and "hairy" roots that synthesized recombinant proteins*

At the end of XX — beginning XXI th century bacterial and viral proteins were shown to synthesize in transgenic plants [37, 38; Table 2]. Antigens synthesized in transgenic plants exhibited immunogenicity [39, 40], so transgenic plants using as a biological system for medical proteins accumulation is of interest in biotechnology research. Plants produced recombinant proteins can be used for the synthesis of valuable compounds for further extraction and also as a so-called "edible" vaccine which provides direct human or animal immunization [41, 42].




***Cichorium intybus* (*a*), *Lactuca sativa* (*b*), *Tragopogon porrifolius* (*c*), *Atremisia tilesii* (*d*) "hairy" root culture carrying human *ifn*-α*2b* gene**

Lettuce is one of a potential candidates for production of "edible" vaccines as used without heat treatment. The results of several studies using these plants for expression of transferred microbial, viral and animal origin genes recently were published. Cheng-Wei Liu et al. [43] constructed transgenic lettuce plants expressing H1N1 influenza surface antigen (neuraminidase) in 0.018–0.045% of total soluble protein. An immune response against the antigen was detected after immunization using transgenic lettuce extracts. The authors identified transgenic plants as a safe and economical system for expression of recombinant proteins for their use as "edible" vaccines.

*A. tumefaciens*-mediated transformation was used to obtain the transgenic lettuce plants carried HIV (human immunodeficiency virus) *gap-gp120* gene [44]. Li et al. [45] demonstrated the feasibility of producing SARS-CoV (respiratory syndrome coronavirus) spike protein S1 in nuclear- and chloroplast-transformed plants, indicating its high potential in development of a safe oral recombinant subunit vaccine against the SARS-CoV in edible plants. Lettuce plants were used for surface antigen HBsAg hepatitis B virus gene transfer [46]. Kanamycin resistant lettuce planti were obtained after explants cocultivation with *A. tumefaciens* C58C1RifR carried *HBsAg* and *nptII* genes; the presence of both genes transferred was confirmed using PCR analysis. Deng et al. [47] introduced the fused gene *O*21-*O*14-*A*21 containing both type O and A FMDV (foot-and-mouth disease virus) epitopes into lettuce. PCR and Southern blot analyses confirmed the integration of the transferred genes to the plant genome. Metabolism of transgenic plants overexpressed *E. coli* asparagine synthetase A gene was investigated. In some transgenic lines the leaf mass increasing, higher asparagine, glutamine, valine, isoleucine and protein levels, lower nitrate contents were demonstrated. In one line overstored inulin was found [48].

Expression of a cholera toxin B subunit-neutralizing epitope of the porcine epidemic diarrhea virus fusion *sCTB-sCOE* gene in transgenic lettuce was demonstrated. Expression level of CTB-COE fusion proteins reached 0.0065% of the total soluble protein in transgenic lettuce leaf tissues [49]. Kim et al. [50] transferred *Escherichia coli* sLTB gene to lettuce. Recombinant sLTB protein accumulated in amount 1.0–2.0% of the total soluble protein.

In our studies *A. tumefaciens* and *A. rhizogenes* were used to obtaine *Cichorium intybus* and *Lactuca sativa* transgenic plants and "hairy" roots carried *esxA*:*fbpB* fused genes of *Mycobacterium tuberculosis* ESAT6:Ag85B antigens [51, 52]. We also transferred human interferon-α2b *ifn*-α*2b* gene in *C. intybus* and *L. sativa* [53, 54] and studied antiviral activity of extracts from transgenic plants and roots [55]. The possibility of *Artemisia tilesii* Ledeb (Aleutian wormwood) genetic transformation using *ifn*-α*2b* gene was studied in our experiments. The frequency of root formation was up to 100% and did not differ in the case of younger or older leaves used as explants (Figure).

Expression of F1-V immunogenic protein of *Yersinia pestis* was detected in transgenic lettuce plants [56]. Mice immunized with lettuce expressing the F1-V antigen developed systemic humoral responses. So, the transgenic plants could be used as a candidate for a plant-based plague vaccine production. Lettuce plants were also used for expression of a cholera toxin B subunit-neutralizing epitope of the porcine epidemic diarrhea virus fusion gene [57, 58]. The expression level of CTB-COE fusion proteins reached 0.0065% of the total soluble protein [58].

Thus, the results of resent investigations testified to efficiency of usage of biotechnological approaches to create plants with novel traits. *A. tumefaciens* and *A. rhizogenes* transformation methods using allows to increase accumulation of valuable compounds in plants traditionally known as a source of

*Table 2.* **Experiments for construction of Compositae transgenic plants and "hairy" roots synthesized recombinant proteins**

| Plant species | Transformation using | Transformation results | References |
|---|---|---|---|
| *Lactuca sativa* | *A. tumefaciens* | Transgenic plants synthesized NA (neuraminidase) protein of H1N1 influenza surface antigen | [43] |
| *Lactuca sativa* | *A. tumefaciens* | Lettuce transgenic plants with HIV *gap-gp120* gene were constructed | [44] |
| *Lactuca sativa* | *A. tumefaciens* | Transgenic plants accumulated SARS-CoV spike protein | [45] |
| *Lactuca sativa* | *A. tumefaciens* | Transgenic lettuce plants carrying hepatitis B virus antigen HBsAg were obtained | [46] |
| *Lactuca sativa* | *A. tumefaciens* | Transformation of lettuce with FMDV epitopes fused gene | [47] |
| *Lactuca sativa* | *A. tumefaciens* | NMR metabolic profiling of transgenic lettuce overexpressing the *E. coli* asparagine synthetase A gene was defined | [48] |
| *Lactuca sativa* | *A. tumefaciens* | A cholera toxin B subunit-neutralizing epitope of the porcine epidemic diarrhea virus fusion gene was transferred in lettuce plants | [49] |
| *Lactuca sativa* | *A. tumefaciens* | *Escherichia coli* heat-labile enterotoxin B subunit gene was transferred in lettuce | [50] |
| *Lactuca sativa* | *A. tumefaciens* | Transgenic lettuce plants carrying *Mycobacterium tuberculosis esxA* and *fbpB* genes were constructed | [51] |
| *Cichorium intybus* | *A. tumefaciens* | Transgenic chicory plants with *Mycobacterium tuberculosis esxA* gene were constructed | [52] |
| *Cichorium intybus* | *A. tumefaciens* | Transformation of chicory plants using human *ifn-α2b* gene | [53] |
| *Lactuca sativa* | *A. rhizogenes* | Lettuce "hairy" roots with human *ifn-α2b* gene were obtained | [54] |
| *Lactuca sativa*<br>*Cichorium intybus* | *A. rhizogenes*<br>*A. tumefaciens* | Antiviral activity of extracts of transgenic chicory and lettuce plants with the human interferon *α2b* gene was studied | [55] |
| *Lactuca sativa* | *A. tumefaciens* | The immunogenic fusion protein F1-V from *Y. pestis* was introduced into lettuce and transgenic lines were developed. Mice immunized with lettuce expressing the F1-V antigen developed systemic humoral responses | [56] |
| *Lactuca sativa* | *A. tumefaciens* | The sCTB gene was transferred to lettuce plants. The expression level of CTB was relatively high (0.24% TSP) in transgenic lettuce | [57] |

biologically active substances. Cultivation of biotechnological "hairy" roots in bioreactors makes it possible to obtain guaranteed friendly raw materials, which is important in consideration of progressive environment pollution. Using of "hairy" roots allows to save natural plant populations and at the same time use plant material for production of high quality plant-derived compounds for medical and agricultural purposes.

Transgenic plants, including edible, are good alternative for synthesis of biologically active compounds. Progress in the plants molecular biology and genetic engineering has lead to the development of approaches for foreign genes high expression including bacterial and viral antigens for vaccine production in plants. Transgenic plants have been demonstrated to be an attractive heterologous expression system for more than 100 recombinant proteins production in the past 10–15 years [58–63]. For example Biolex company has commercialized recombinant interferon production using transgenic duckweed plants [64, 65]. Heterologous proteins such as therapeutics, antibodies, vaccines and enzymes for pharmaceutical and industrial applications may be successfully expressed also in edible or medical Compositae plants. These plants can be promising alternatives to microbial synthesis of BAS due to product safety, cost-effective biomanufacturing, recombinant proteins post-translational modifications in plant cells production system. Development of technologies for production of plant derived recombinant proteins can be used in medicine for the creation of "herbal" drugs for the prevention and treatment of numerous diseases.

## REFERENCES


1. *Li Y., Huang H., Wu Y.L.* Qinghaosu (artemisinin)—a fantastic antimalarial drug from a traditional Chinese medicine. In: Liang X. T., Fang W. S. (eds). *Medicinal Chemistry of Bioactive Natural Products. Wiley, New York*. 2006, 183–256.
2. *Weathers P. J., Arsenault P. R., Covello P. S., McMickle A., Teoh K. H., Reed D. W.* Artemisinin production in *Artemisia annua*: studies *in planta* and results of a novel delivery method for treating malaria and other neglected diseases, *Phytochem. Rev*. 2011, 10(2), 173–183. PMID:21643453.
3. *Chen D.H., Liu C.J., Ye H.C., Guo-Feng Li, Ben-Ye Liu, Yu-Ling Meng, Xiao-Ya Chen.* Ri-mediated transformation of *Artemisia annua* with a recombinant farnesyl diphosphate synthase gene for artemisinin production. *Plant Cell Tis. Org. Cult.* 1999, 57 (3), 157–162. doi 10.1023/A:1006326818509.
4. *Chen D.H., Ye H.C., Li G.F.* Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. *Plant Sci.* 2000, 155(2), 179–185. PMID:10814821.
5. *Han J.L., Liu B.Y., Ye H.C., Hong Wang, Zhen-Qiu Li, Guo-Feng Li.* Effects of overexpression of the endogenous farnesyl diphosphate synthase on the artemisinin content in *Artemisia annua* L. *J. Integr. Plant Biol.* 2006, 48(4), 482–487. doi: 10.1111/j.1744-7909.2006.00208.x.
6. *Banyai W., Kirdmanee C., Mii M.,Supaibulwatana K*. Overexpression of farnesyl pyrophosphate synthase (FPS) gene affected artemisinin content and growth of *Artemisia annua* L. *Plant Cell Tiss. Org. Cult*. 2010, 103(2), 255–265. doi: 10.1007/s11240-010-9775-8.
7. *Geng S., Ma M., Ye H.C., Liu Ben-ye, Li Guo-feng, Chong Kang.* Effects of *ipt* gene expression on the physiological and chemical characteristics of *Artemisia annua* L. *Plant Sci.* 2001, 160(4), 691–698. doi: 10.1016/S0168-9452(00)00453-2.
8. *Alam P., Abdin M.Z.* Over-expression of HMG-CoA reductase and amorpha-4,11-diene synthase genes in *Artemisia annua* L. and its influence on artemisinin content. *Plant Cell Rep.* 2011, 30(10), 1919–1928. doi: 10.1007/s00299-011-1099-6.
9. *Chen J. L., Fang H. M., Ji Y. P.. Pu G. B., Guo Y. W., Huang L. L., Du Z. G., Liu B. Y., Ye H. C., Li G. F., Wang H.* Artemisinin biosynthesis enhancement in transgenic *Artemisia annua* plants by downregulation of the beta-caryophyllene synthase gene. *Planta Med*. 2011, 77(15), 1759–1765. PMID:21509717
10. *Lu X., Shen Q., Zhang L., Fangyuan Zhang, Weimin Jiang, Zongyou Lv, Tingxiang Yan, Xueqing Fu, Guofeng Wang, Kexuan Tang.* Promotion of artemisinin biosynthesis in transgenic *Artemisia annua* by overexpressing *ADS*, *CYP71AV1* and *CPR* genes. *Ind. Crop Prod.* 2013, V. 49, 380–385. doi: 10.1016/j.indcrop.2013.04.045.
11. *Tang K.X., Jiang W.M., Lu X*. Overexpression *AaWRYK1* gene increased artemisinin content in *Artemisia annua* L. Shanghai Jiao Tong University, China. *Patent CN201210249469*. X, 2012.
12. *Yueyue Wang, Fuyuan Jing, Shuoye Yu, Yunfei Chen, Tao Wang, Pin Liu, Guofeng Wang, Xiaofen Sun, Kexuan Tang.* Co-overexpression of the *HMGR* and *FPS* genes enhances artemisinin content in *Artemisia annua* L. *J. Med. Plants Res.* 2011, 5(15), 3396–3403.
13. *Wang H., Ge L., Ye H.C., Chong K., Liu B. Y., Li G. F*. Studies on the effects of *fpf1* gene on *Artemisia annua* flowering time and on the linkage between flowering and artemisinin biosynthesis. *Planta Med.* 2004, 70(4), 347–352. PMID:15095151.
14. *Wang H., Liu Y., Chong K., Liu B. Y., Ye H. C., Li Z. Q., Yan F., Li G. F*. Earlier flowering induced by over-expression of CO gene does not accompany increase of artemisinin biosynthesis in *Artemisia annua*. *Plant Biol.* 2007, 9(3), 442–446. PMID:17099845.
15. *Giri A., Ravindra S. T., Dhingra V., Narasu M. L*. Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy root and artemisinin production in *Artemisia annua*. *Current Sci.* 2001, 81(4), 4–25.
16. *Weathers P.J., Bunk G., McCoy M.C*. The effect of phytohormones on growth and artemisinin production in *Artemisia annua* hairy roots. *In Vitro Cell Dev. Biol. Plant*. 2005, 41(1), 47–53. doi:10.1079/IVP2004604.
17. *Wyslouzil B.E., Waterbury R.G., Weathers P.J*. The growth of single roots of *Artemisia annua* in nutrient mist reactors. *Biotechnol. Bioeng.* 2000, 70(2), 143–150. PMID:10972925.
18. *Kim Y. J., Weathers P. J., Wyslouzil B. E*. Growth of *Artemisia annua* hairy roots in liquidand gas-phase reactors. *Biotechnol. Bioeng*. 2002, 80(4), 454–464. PMID:12325154.
19. *Souret F. F., Kim Y., Wyslouzil B. E., Wobbe K. K., Weathers P. J*. Scale-up of *Artemisia annua* L. hairy root cultures produces complex patterns of terpenoid gene expression. *Biotechnol. Bioeng*. 2003, 83(6), 653–667. PMID:12889030.
20. *Kim Y. J., Weathers P. J., Wyslouzil B. E*. Growth dynamics of Artemisia annua hairy roots in three culture systems. *Biotechnol. Bioeng.* 2003, 83(4), 428–443. PMID:12800137.
21. *Mannan A., Shaheen N., Arshad W., Qureshi R. A., Muhammad Zia, Bushra Mirza.* Hairy roots induction and artemisinin analysis in *Artemisia dubia* and *Artemisia indica*. *Afr. J. Biotechnol.* 2008, 7(18), 3288–3292.

22. *Sujatha G., Zdravkovic-Korac S., Calic D., Flamin G., Ranjitha Kumari B. D.* High-efficiency *Agrobacterium rhizogenes*-mediated genetic transformation in *Artemisia vulgaris*: Hairy root production and essential oil analysis. *Industrial Crops and Prod.* 2013, V.44, P. 643–652. doi: 10.1016/j.indcrop.2012.09.007.
23. *Nin S., Bennici G., Roselli D., Mariotti D., Schiff S., Magherini R. Agrobacterium*-mediated transformation of *Artemisia absinthium* L. (wormwood) and production of secondary metabolites *Plant Cell Rep*. 1997, 16(10), 725–730. 10.1007/s002990050310.
24. *Eun-Hyang Han, Ji-Sun Lee, Jae-Woong Lee* Transgenic lettuce expressing chalcone isomerase gene of chinese cabbage increased levels of flavonoids and polyphenols. *Kor. J. Hort. Sci. Technol*. 2011, 29(5), 467–473.
25. *Sobolev A. P., Segre A. L., Giannino D., Mariotti D., Nicolodi C., Brosio E., Amato M. E.* Strong increase of foliar inulin occurs in transgenic lettuce plants (*Lactuca sativa* L.) overexpressing the Asparagine Synthetase A gene from *Escherichia coli*. *J. Agric. Food Chem*. 2007, 55(26), 10827–10831. PMID:18044837.
26. *Vijn I., van Dijken A., Sprenger N., van Dun K., Weisbeek P., Wiemken A., Smeekens S.* Fructan of the inulin neoseries is synthesized in transgenic chicory plants (*Cichorium intybus* L.) harbouring onion (*Allium cepa* L.) fructan:fructan 6G-fructosyltransferase. *Plant J*. 1997, 11(3), 387–398. PMID:9107030.
27. *George J., Bais H , Ravishankar, G. A.* Production of esculin by hairy root cultures of chicory (*Cichorium intybus* L.cv. Lucknow local). *Indian J. Exp. Biol*. 1999, V. 37, P. 269–273.
28. *Bais H. P., Sudha G., Ravishankar G. A.* Enhancement of growth and coumarin production in hairy root cultures of *Cichorium intybus*, L. cv. Lucknow Local (Witloof Chicory) under the influence of fungal elicitors. *J. Biosci. Bioeng*. 2000, 90(6), 640–645. PMID:16232926.
29. *Bais H. P., Suresh B., Raghavarao K., Ravishankar G. A.* Performance of hairy root cultures of *Cichorium intybus* L. in bioreactors of different configurations. *In Vitro Cell Dev. Biol. Plant*. 2002, 38(6), 573–580. doi: 10.1079/IVP2002334.
30. *Matvieieva N. A., Tsygankova V. A., Chapkevych S. O., Kuchuk M. V., Ponomarenko S. P.* Using of growth regulators for intensification of biomass growth and increasing of fructan content in chicory "hairy" root culture, *Visnyk Ukr. tov. henetykiv ta selektsioneriv*. 2012, 10(2), 269–278 (In Ukrainian).
31. *Weremczuk-Jeżyna I., Kisiel W., Wysokińska H*. Thymol derivatives from hairy roots of *Arnica montana*. *Plant Cell Rep*. 2006, 25(9), 993–996. PMID:16586074.
32. *Długosz M., Wiktorowska E., Wiśniewska A., Pączkowski C*. Production of oleanolic acid glycosides by hairy root established cultures of *Calendula officinalis* L. *Acta Biochim Pol*. 2013, 60(3), 467–473. PMID:24040627.
33. *Tada H., Murakami Y., Omoto T.* Rosmarinic acid and related phenolics in hairy root cultures of *Ocimum basilicum*. *Phytochem*., 1996, 42(2), 431–434. doi: 10.1016/0031-9422(96)00005-2
34. *Ray S., Ghosh B., Sen S., Jha S*. Withanolide production by root cultures of *Withania somnifera* transformed with *Agrobacterium rhizogenes*. *Planta Med*. 1996, 62(6), 571–573. PMID:17252504.
35. *Bandyopadhyay M., Jha S., Tepfer D.* Changes in morphological phenotypes and withanolide composition of Ri-transformed roots of Withania somnifera. Plant Cell Rep., 2007, 26(5), 599–609. PMID:17103214.
36. *Matvieieva N. A.* Construction of *Tragopogon porrifolius* and *Altaea officinalis* "hairy" root cultures using *Agrobacterium rhizogenes*, *Visnyk Ukr. tov. henetykiv ta selektsioneriv*, 2012, 10(2), 262–268. (In Ukrainian).
37. *Daniell H., Lee S. B., Panchal T., Wiebe P. O.* Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts. *J. Mol. Biol*. 2001, 311(5), 1001–1009. PMID:11531335.
38. *Franconi R., Di Bonito P., Dibello F., Accardi L., Muller A., Cirilli A., Simeone P., Donà M.G., Venuti A., Giorgi C*. Plant-derived human papillomavirus 16 E7 oncoprotein induces immune response and specific tumor protection. *Cancer Res*. 2002, 62(13), 3654–3658. PMID:12097270.
39. *Kong Q., Richter L., Yang Y.F., Arntzen C. J., Mason H. S., Thanavala Y.* Oral immunization with hepatitis B surface antigen expressed in transgenic plants. *Proc. Natl. Acad. Sci. USA*. 2001, 98(20), 11539–11544.
40. *Thanavala Y., Mahoney M., Pal S., Scott A., Richter L., Nachimuthu Natarajan, P. Goodwin, Arntzen C. J., Mason H. S*. Immunogenicity in humans of an edible vaccine for hepatitis B. *Proc. Natl. Acad. Sci. USA*. 2005, 102(9), 3378–3382. doi: 10.1073/pnas.0409899102.
41. *Walmsley A. M., Arntzen C. J*. Plant cell factories and mucosal vaccines. *Curr. Opin. Biotechnol*. 2003, 14(2), 145–150. PMID:2732315.
42. *Joensuu J.J., Niklander-Teeri V., Brandle J. E.* Transgenic plants for animal health: plant-made vaccine antigens for animal infectious disease control. *Phytochem. Rev*. 2008, 7(3), 553–577. doi: 10.1007/s11101-008-9088-2.
43. *Cheng-Wei Liu, Jeremy J.W. Chen, Chia-Chen Kang, Chia-Hui Wu, Jinn-Chin Yiu.* Transgenic lettuce (*Lactuca sativa* L.) expressing H1N1 influenza surface antigen (neuraminidase). *Sci. Horticult*. 2012, V. 139, P. 8–13. doi: 10.1016/j.scienta.2012.02.037.
44. *Jing J., Ji J., Wang G.* Establishment of genetic transformation system of lettuce by

*Agrobacterium tumefaciens* and introduction of HIV gap-gp120 gene. *Tianjin Agric. Sci*. 2007, 13(1), 14–17.

45. *Li H. Y., Ramalingam S., Chye M. L.* Accumulation of recombinant SARS-CoV spike protein in plant cytosol and chloroplasts indicate potential for development of plant-derived oral vaccines. *Exp. Biol. Med*. 2006, 231(8), 1346–1352. PMID:16946403.
46. *Marcondes J., Hansen E.* Transgenic lettuce seedlings carrying hepatitis B virus antigen HBsAg. *Braz. J. Infect. Dis*. 2008,12(6), 469–471. PMID:19287831.
47. *Deng Xiao-Li, Chang Jing-Ling, He Jie, He Guang-Cun* Transformation of lettuce with FMDV epitopes fused gene mediated by *Agrobacterium*, *Plant Sci. J*. 2006, 4(5), 476–479.
48. *Sobolev A. P., Testone G., Santoro F., Nicolodi C., Iannelli M. A., Amato M. E., Ianniello A., Brosio E., Giannino D., Mannina L.* Quality traits of conventional and transgenic lettuce (Lactuca sativa L.) at harvesting by NMR metabolic profiling. *J. Agric Food Chem*. 2010, 58(11), 6928–6936. doi: 10.1021/jf904439y.
49. *Huy N. X., Yang M. S., Kim T. G.* Expression of a cholera toxin B subunit-neutralizing epitope of the porcine epidemic diarrhea virus fusion gene in transgenic lettuce (*Lactuca sativa* L.). *Mol. Biotechnol*. 2011, V. 48, P. 201–209. doi: 10.1007/s12033-010-9359-1.
50. *Kim T. G., Kim M. Y., Kim B. G., Kang T. J., Kim Y. S., Jang Y. S., Arntzen C. J., Yang M. S.* Synthesis and assembly of Escherichia coli heat-labile enterotoxin B subunit in transgenic lettuce (Lactuca sativa ). *Protein Expr. Purif*. 2007, 51(1), 22–27.
51. *Matvieieva N. A., Vasylenko M. Y., Shakhovsky A. M., Kuchuk N. V.* Agrobacterium-mediated transformation of lettuce (*Lactuca sativa* L.) with vectors bearing genes of bacterial antigenes from *Mycobacterium tuberculosis*. *Tsitol. Genet*. 2009, 43(2), 27–32. PMID:19938623.
52. *Matvieieva N. A., Vasylenko M. Y., Shahovsky A. M., Bannykova M. O., Kvasko O. Y., Kuchuk N. V*. Effective Agrobacterium-mediated transformation of chicory (*Cichorium intybus* L.) with *Mycobacterium tuberculosis* antigene ESAT6. *Cytol. Genet*. 2011, 45(1), 7–12. doi: 10.3103/S0095452711010038.
53. *Matvieieva N. A., Shachovsky A. M., Gerasymenko I. M., Kvasko O. Y., Kuchuk N. V.* Agrobacterium-mediated transformation of *Cichorium intybus* L. with interferon-a2b gene. *Biopolymers and Cell*. 2009, 25(2), 120–126.
54. *Matvieieva N. A., Shahovsky A. M., Kuchuk M. V.* Features of lettuce transgenic plants with ifnα2b gene regenerated after Agrobacterium rhizogenes-mediated transformation. *Cytol. Genet*. 2012, 46 (3), 150–154. PMID:22856143.
55. *Matvieieva N. A., Kudryavets Yu. I., Likhova A. A., Shakhovskij A. M., Bezdenezhnykh N. A., Kvasko E. Yu*. Antiviral activity of extracts of transgenic chicory and lettuce plants with the human interferon α*2b* gene. *Cytol. Genet*. 2012, 46 (5), 285–290. doi:10.3103/S0095452712050076.
56. *Rosales-Mendoza S., Soria-Guerra R. E., Moreno-Fierros L., Alpuche-Solís A. G.* Expression of an immunogenic F1-V fusion protein in lettuce as a plant-based vaccine against plague. *Planta*. 2010, 232(2), 409–416. doi: 10.1007/s00425-010-1176-z.
57. *Young-Sook Kim, Bang-Geul Kim, Tae-Geum Kim, Tae-Jin Kang, Moon-Sik Yang.* Expression of a cholera toxin B subunit in transgenic lettuce (*Lactuca sativa* L.) using Agrobacterium-mediated transformation system. *Plant Cell Tiss. Organ Cult*. 2006, 87(2), 203–210. doi:10.1007/s11240-006-9156-5.
58. *Siddharth Tiwari, Praveen C. Verma, Pradhyumna K. Singh, Rakesh Tuli.* Plants as bioreactors for the production of vaccine antigens. *Biotechnol. Adv*. 2009, 27, 449–467 doi:10.1016/j.biotechadv.2009.03.006.
59. *Orzáez D., Granell A., Blázquez M. A.* Manufacturing antibodies in the plant cell. *Biotechnol. J*. 2009, 4(12), 1712–1724. doi: 10.1002/biot.200900223.
60. *Daniell H., Nameirakpam D. Singh, Mason H., Streatfield S. J*. Plant-made vaccine antigens and Biopharmaceuticals. *Trends in Plant Sci.* 2009, 14(12), 669–679. doi: 10.1016/j.tplants.2009.09.009.
61. *Sharma A. K. Sharma M. K.* Plants as bioreactors: Recent developments and emerging opportunities. *Biotechnol. Adv.* 2009, V. 27, P. 811–832. doi:10.1016/j.biotechadv.2009.06.004.
62. *Skarjinskaia M., Ruby K, Araujo A., Taylor K., Gopalasamy-Raju V., Musiychuk K., Chichester J. A., Palmer G. A., de la Rosa P., Mett V., Ugulava N., Streatfield S. J., Yusibov V*. Hairy roots as a vaccine production and delivery system. *Adv. Biochem. Eng. Biotechnol.* 2013, V. 134, P. 115–134. doi: 10.1007/10_2013_184.
63. *Huang T. K., McDonald K. A.* Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. *Biotechnol. Adv.* 2012, 30 (2), 398–409. doi: 10.1016/j.biotechadv.2011.07.016.
64. *Fischer R., Schillberg S., Hellwig S., Twyman R. M., Drossard J*. GMP issues for recombinant plant-derived pharmaceutical proteins. *Biotechnol. Adv*., 2012, 30 (2), 434–439. doi: 10.1016/j.biotechadv.2011.08.007.
65. *De Leede L. G., Humphries J. E., Bechet A. C., Van Hoogdalem E. J., Verrijk R., Spencer D. G.* Novel controlled-release Lemna-derived IFN-alpha2b (Locteron): pharmacokinetics, pharmacodynamics, and tolerability in a phase I clinical trial. *J. Interferon Cytokine Res*. 2008, 28 (2), 113–122. doi: 10.1089/jir.2007.0073.

# ВИКОРИСТАННЯ *Agrobacterium*-ОПОСЕРЕДКОВАНОЇ ТРАНСФОРМАЦІЇ У БІОТЕХНОЛОГІЇ РОСЛИН РОДИНИ COMPOSITAE. II. СИНТЕЗ БІОЛОГІЧНО АКТИВНИХ СПОЛУК У ТРАНСГЕННИХ РОСЛИНАХ ТА «БОРОДАТИХ» КОРЕНЯХ

*Н. А. Матвєєва*

Інститут клітинної біології і генетичної інженерії НАН України, Київ

*E-mail: joyna56@gmail.com*

Розглянуто дані літератури і власних досліджень автора щодо створення «бородатих» коренів і трансгенних рослин родини *Compositae* шляхом трансформації бактеріями *Agrobacterium rhizogenes* та *A. tumefaciens* з метою одержання біологічно активних сполук, у тому числі рекомбінантних протеїнів. Наведено результати генетичної трансформації рослин *Cichorium intybus*, *Lactuca sativa, Artemisia annua*, *Artemisia vulgaris, Calendula officinalis, Withania somnifera*, а також досліджень рівня синтезу та накопичення артемізину, флавоноїдів, поліфенолів, фруктанів та інших сполук у трансгенних рослинах і «бородатих» коренях. Отримані дані показують, що трансформовані рослини та «бородаті» корені рослин родини Compositae здатні синтезувати рекомбінантні сполуки відповідно до перенесених генів. Розроблення технологій одержання рекомбінантних протеїнів, а також інших біологічно активних сполук, що продукуються у трансгенних коренях та рослинах родини складноцвітих, може набути застосування у медицині та ветеринарії для створення «рослинних» ліків з метою профілактики та лікування низки захворювань.

***Ключові слова:*** Compositae, *Agrobacterium tumerfaciens*, *Agrobacterium rhizogenes*, трансгенні рослини, «бородаті» корені, біологічно активні сполуки, рекомбінантні протеїни.

# ИСПОЛЬЗОВАНИЕ *Agrobacterium*-ОПОСРЕДОВАННОЙ ТРАНСФОРМАЦИИ В БИОТЕХНОЛОГИИ РАСТЕНИЙ СЕМЕЙСТВА COMPOSITAE. II. СИНТЕЗ БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ В ТРАНСГЕННЫХ РАСТЕНИЯХ И «БОРОДАТЫХ» КОРНЯХ

*Н. А. Матвеева*

Институт клеточной биологии и генетической инженерии НАН Украины,

*E-mail: joyna56@gmail.com*

Рассмотрены данные литературы и собственных исследований автора по созданию «бородатых» корней и трансгенных растений семейства Compositae путем трансформации бактериями *Agrobacterium tumefaciens* и *A. rhizogenes* с целью получения биологически активных соединений, включая рекомбинантные протеины. Приведены результаты генетической трансформации растений *Cichorium intybus, Lactuca sativa, Artemisia annua, Artemisia vulgaris, Calendula officinalis, Withania somnifera*, а также исследований уровня синтеза и накопления артемизина, флавоноидов, полифенолов, фруктанов и других соединений в трансгенных растениях и «бородатых» корнях. Полученные данные показывают, что трансформированные растения и «бородатые» корни растений, относящихся к семейству Compositae, могут синтезировать рекомбинантные протеины соответственно перенесенным генам. Разработка технологий получения рекомбинантных протеинов, а также других биологически активных соединений, продуцируемых в трансгенных корнях и растениях семейства сложноцветных, может найти применение в медицине и ветеринарии для создания «растительных» лекарств с целью профилактики и лечения ряда заболеваний.

***Ключевые слова:*** Compositae, *Agrobacterium tumerfaciens, Agrobacterium rhizogenes*, трансгенные растения, «бородатые» корни, биологически активные соединения, рекомбинантные протеины.