Agrobacterium-MEDIATED TRANSFORMATION OF COMPOSITAE PLANTS. I. CONSTRUCTION OF TRANSGENIC PLANTS AND «HAIRY» ROOTS WITH NEW PROPERTIES

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Received 12.01.2015

The review explores some of the recent advances and the author’s own researchs concerning biotechnological approaches for Agrobacterium tumefaciens- and A. rhizogenes-mediated transformation of Compositae family plants. This paper reviews the results of genetic transformation of Compositae plants, including edible (Cichorium intybus, Lactuca sativa), oil (Helianthus annuus), decorative (Gerbera hybrida), medical (Bidens pilosa, Artemisia annua, Artemisia vulgaris, Calendula officinalis, Withania somnifera etc.) plant species. Some Compositae genetic engineering areas are considered including creation of plants, resistant to pests, diseases and herbicides, to the effect of abiotic stress factors as well as plants with altered phenotype. The article also presents the data on the development of biotechnology for Compositae plants Cynara cardunculus, Arnica montana, Cichorium intybus, Artemisia annua “hairy” roots construction.

Key words: Compositae, Agrobacterium tumefaciens, Agrobacterium rhizogenes, “hairy” roots.

Eighties of the 20th century were the beginning of active development and widespread adoption of genetically engineered plants. The development of methods of gene cloning and methods of transferring of genetic information into a plant genome served as background and stimulation of this process. The study concerning Genetic Engineering is dedicated to basic research of the functioning of the transferred genes and practical use of new biotechnological approaches. First efforts were directed at developing of biotechnology to obtain plants with novel features, such as resistance to pests, diseases and to abiotic stresses. These plants should also provide an increased synthesis of biologically active compounds and recombinant proteins. Transformation using Agrobacterium tumefaciens and A. rhizogenes is a simple and effective method of construction of biotechnological plants (plants with transformed genome). These soil pathogenic bacteria are able to transfer part of their DNA, Ti- or Ri-plasmids to plant genome [1–3]. The using of foreign genes transferring technology into plant DNA is possible due to natural properties of phytopathogenic bacteria and progress in cloning technology. Transformation using A. tumefaciens and development of methods of shoot regeneration for certain species allowed to obtain transgenic plants for a relatively short period of time. This method is usually used to create new forms of crops resistant to diseases and pests, plants which synthesize recombinant proteins (called “edible” vaccines), decorative plants with new phenotype. The special attention has to be paid to the use of bacteria A. rhizogenes for genetic transformation and construction of “hairy” roots which are characterized by genetic stability [4] and grow on medium without growth regulators. Growing such roots in bioreactors is not expensive and can be used to produce bioactive compounds of plant origin [5–9]. Nowadays, the system of genetic transformation and creation of transgenic roots for a large number of plants have been developed. The possibility to increase the
content of bioactive compounds which are naturally synthesized in an original plants has been shown [10–16].

Agrobacterium-mediated transformation is also used to create Compositae plants with a new characteristics. Compositae family includes about 2,000 species.

Among them there are medicinal (eg, Arnica, Inula, Calendula, Tragopogon, Tussilago, Artemisia, Sylphium, Stevia, Echinacea), oil (Helianthus), decorative (Gerbera, Cosmos, Dahlia, Bellis, Chrysanthemum, Echinacea) and edible (Lactuca, Cichorium, Cynara) plants. Compositae family plants are used in traditional medicine, they reveal antiradical and antioxidant activity [17–19], synthesize compounds with anti-inflammatory [20], hepatoprotective [21–23], cytotoxic [24–26], antiparasitic [27], antimicrobial [28] and immunomodulatory [29] properties. Many genetic engineering experiments on the Compositae plants were carried out to improve their resistance to diseases, pests and abiotic stress factors, to increase the content of important compounds, to select the plants with altered phenotype. In addition, the study was designed to create plants that synthesize recombinant proteins with therapeutic properties. The following table provides information on the most significant studies on Agrobacterium-mediated transformation of Compositae family plants.

### Optimization of conditions of genetic transformation

The first investigations on genetic transformation of Compositae plants were dated in the late eighties of the 20th century. They were aimed at fundamental development of the transformation system using bacteria A. tumefaciens and A. rhizogenes. It is known that the efficiency of transformation depends on the complex of factors which are associated with both virulent activity of agrobacteria strains and morphological, physiological and characteristics of transformed plants. Selection of transgenic plants (correct choice of selective and reporter genes) is an important step of transformation. Therefore, the definition of «critical» i.e. principal for plant species parameters is essential to obtain transgenic plants or roots. Considering all above mentioned factors, it is necessary to define the optimal type of explants, transforming conditions, using different strains of agrobacteria, reporter and selective genes etc. The greatest amount of research has been aimed at developing of transformation protocols for the plant species, which are very important for agriculture and medicine such as Lactuca sativa, Artemisia annua, Cichorium intybus and Helianthus annuus. Lettuce and chicory plants are used for food; chicory, thanks its compounds with therapeutic properties, is used as a base for certain medicines. That is why these species cultivated in vitro have been studied in detail. For example, to obtain transgenic plants of lettuce the effect of plant genotype, the concentration of bacteria in suspension for transformation and a period of transformation on the efficiency of transformation were determined [61]. Optimal conditions for transformation of lettuce in particular the time of cocultivation with bacteria were defined in [72].

Efficiency of mannose phosphate isomerase, neomycin phosphate transferase II, beta glucuronidase selective genes and reporter gus gene usage to select L. sativa and C. intybus transgenic plants was investigated [39, 40, 72].

The influence of plant genotype on the regenerative ability of three C. intybus (Witloof, Melci, Hera) cultivars and obtaining of transgenic plants using A. tumefaciens were studied [38]. We obtained L. sativa and C. intybus var foliosum transgenic roots using A. rhizogenes-mediated transformation method (wild strain A4) and defined the conditions of shoot regeneration from “hairy” roots. Formation of transgenic shoots on the roots of chicory was occurred spontaneously in culture medium without hormones under lighting conditions whereas lettuce regeneration was possible only after cultivation on the medium which included growth regulators such as kinetin and α-naphthylacetic acid (Figure). The rolB gene of A. rhizogenes was detected in regenerated plants as well as in “hairy” roots.

Artemisia is another species of Compositae family. It includes herbaceous plants which are common in Europe, America, Central Asia and North Africa.

The common wormwood (A. vulgaris L.), annual mugwort (A. annua), absinthium (A. absinthium), tarragon (A. dracunculus) and other species belong to this family. These plants are used in official and alternative medicine, so there is a great interest in these species, especially in China and India, where this plant material is widely used in medical practice. A. rhizogenes and A. tumefaciens were used to perform genetic transformation of Artemisia sp. As it was shown in [49] A. annua was transformed by the A. rhizogenes LBA 9402 strain, transgenic roots were obtained and the
**Agrobacterium-mediated transformation of plants of Compositae family**

| Species                  | Usage          | The way of transformation | The result of transformation                                                                 | References |
|--------------------------|----------------|----------------------------|------------------------------------------------------------------------------------------------|------------|
| *Arnica montana* L.     | pharmaceutical | *A. rhizogenes*            | Transgenic roots were obtained                                                                  | [30]       |
| *Arnica montana* L.     | pharmaceutical | *A. rhizogenes*            | The light influence on the synthesis of thymol derivates in transgenic roots was investigated   | [11]       |
| *Dahlia pinnata*        | decorative     | *A. tumefaciens*           | Transformation conditions were optimized                                                         | [31]       |
| *Gerbera hybrida*       | decorative     | *A. tumefaciens*           | Plants with altered pigmentation were obtained                                                  | [32]       |
| *Gerbera hybrida*       | decorative     | *A. tumefaciens*           | Transformation conditions were optimized                                                         | [33], [34] |
| *Gerbera jemosonii*     | decorative     | *A. tumefaciens*           | Transformation conditions were optimized                                                         | [35]       |
| *Gerbera hybrida*       | decorative     | *A. tumefaciens*           | Gene of resistance to tomato spotted wilt virus was transferred into plants                     | [36]       |
| *Cynara carduncuus*     | edible         | *A. tumefaciens*           | *gus*-positive callus was obtained                                                              | [37]       |
| *Cichorium intybus*     | edible         | *A. tumefaciens*           | Transformation conditions were optimized                                                         | [38], [39], [40] |
| *Cichorium intybus*     | edible         | *Agrobacterium rhizogenes* | Regenerated plants with early flowering were obtained                                            | [41]       |
| *Cichorium intybus*     | edible         | *Agrobacterium rhizogenes* | The influence of transformation on phenotype altering was defined                               | [42], [43] |
| *Cichorium intybus*     | edible         | *Agrobacterium tumefaciens*| Plants resistant to chlorine sulfon were obtained                                               | [44]       |
| *Cichorium intybus*     | edible         | *Agrobacterium tumefaciens*| The plants resistant to salinity after their transformation by gene *AtNHX1* gene were obtained  | [45]       |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium rhizogenes* | The conditions of transformation were optimized                                                 | [46]       |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium rhizogenes* | The conditions of cultivation of transgenic roots to increase arte-misinin synthesis were defined| [47]       |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium rhizogenes* | The conditions of transformation were optimized, the efficiency of usage of different strains of bacteria for transformation was defined. | [48], [49], [50] |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium tumefaciens*| The plants with altered flowering time were obtained                                             | [51]       |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium tumefaciens*| An effective transformation protocol was developed                                              | [52]       |
| *Artemisia dubia* and *Artemisia indica* | pharmaceutical | *Agrobacterium rhizogenes* | The conditions of growing and synthesis of arte-misinin in transgenic roots were investigated | [53]       |
| *Artemisia annua*       | pharmaceutical | *Agrobacterium tumefaciens*| The conditions of transformation were optimized with the usage of the different strains of agrobacteria | [54], [55] |
| Species                  | Usage      | The way of transformation | The result of transformation                                                                 | References |
|-------------------------|------------|---------------------------|-----------------------------------------------------------------------------------------------|------------|
| Artemisia vulgaris      | pharmaceutical | Agrobacterium rhizogenes | The conditions of transformation were optimized with the usage of the different strains, different explants and under different cultivation conditions | [56]       |
| Artemisia absinthium    | pharmaceutical | Agrobacterium rhizogenes | The culture of transgenic roots was obtained, the production of secondary metabolites was defined | [57]       |
| Taraxacum platycarpum  | pharmaceutical | Agrobacterium rhizogenes | The changes of morphology after transformation were described                               | [58]       |
| Inula helenium          | edible      | Agrobacterium rhizogenes  | The conditions of transformation were optimized                                               | [59]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | Expression of ABF3 gene of Arabidopsis plants increased resistance to drought and cold       | [60]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | The conditions of transformation were optimized                                               | [61]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | Ipt gene was used under the control of SAG12 promoter to prevent aging leaves                 | [62]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | The genes that determine resistance to destruction by insects (pta, ct, cgrp) were transferred | [63], [64] |
| Lactuca sativa          | edible      |                          | The plants resistant to the herbicide (bar-gene) were obtained                              | [65]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | The increase of resistance to abiotic stresses (drought, low temperature) using ABF3, P5CS genes was determined | [66], [67], [68] |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | The transgenic seed was obtained in vitro                                                   | [69]       |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | Plant genes that ensure the resistance to phytovirus (coat protein genes) were integrated into plant genome | [70], [71] |
| Lactuca sativa          | edible      | Agrobacterium tumefaciens | Efficiency of transformation and methods of selection of transgenic plants (genes of mannose phosphate isomerase, neomycin phosphate transferase II) were defined | [72]       |
| Centaurea montana       | pharmaceutical | Agrobacterium tumefaciens | The conditions of transformation were optimized                                               | [73]       |
| Bidens pilosa           | pharmaceutical | Agrobacterium tumefaciens | The conditions of transformation were optimized, the gene of halcon synthase gene was transferred | [74]       |
| Tanacetum ciner-artifolium |             | Agrobacterium tumefaciens | The conditions of transformation were optimized (selection in the presence of hygromycin)     | [75]       |
| Helianthus annuus × Helianthus tuberosus | oil       | Agrobacterium rhizogenes | The method for hybrids rooting was developed                                                  | [76]       |
| Helianthus annuus       | oil        | Agrobacterium tumefaciens | The efficiency of transformation with the usage of different genotypes and types of explant was compared | [77], [78], [79], [80] |
possibility of spontaneous regeneration of shoots from the roots on non-hormonal medium MC was revealed. The efficiency of usage of *A. rhizogenes* A4, 15834, K599, LBA 9402, 9365, 9340 for *Artemisia* plants transformation was estimated [48]. The authors also determine the efficiency of acetosyringon addition to the culture medium during cultivation of agrobacteria or to the medium during explants co-cultivation with bacterial suspension. The genetic transformation of *A. annua* using *A. rhizogenes* LBA 9402 was carried out, the transfer of TL-DNA (*rol* gene) of agrobacterial plasmid was confirmed by PCR analysis [50]. Since the purpose of this study was to create transgenic roots which are characterized by rapid growth and high accumulation of artemisinin, the authors found out the influence of temperature, pH, medium composition and carbon source on “hairy” roots growth. The effect of agrobacteria strains used (*A. rhizogenes* LBA 9402, LBA 920, LBA 301, MTCC 532, NRRL B193 and A4) on the efficiency of *A. annua* transformation was studied. The most suitable type of explant (leaf, stem, petiole) was also revealed [56]. *Artemisia dubia, A. indica, A. absinthium, A. vulgaris* “hairy” root cultures were obtained using *A. rhizogenes*-mediated transformation method [53, 56, 57]. We firstly transformed *A. tilesii* Ledeb (Aleutian wormwood) plants using efficient transformation protocol. We obtained the transgenic roots with the frequency 100% (Figure) under optimized conditions of co-cultivation with *A. rhizogenes* A4. Transgenic *A. annua* plants were obtained using *A. tumefaciens*. The protocol of *A. annua* transformation [52] was optimized. It was shown that this process was effected by *A. tumefaciens* strain, plant genotype, usage of acetosyringon, the period of co-cultivation with bacteria etc. The similar studies with the usage of different strains of agrobacteria (LBA4404, GV1301, AGL1, EHA105) and different conditions of transformation (age of explants, the method of inoculation of bacteria, duration and conditions of co-cultivation of explants with bacteria) were carried out in [54] and [55]. Nowadays number of Compositae plant species which are used for genetic transformation significantly expands. It includes for instance *Cynara cardunculus* [37], *Gerbera jemosonii* [35], *Inula helenium* [59], *Centaurea montana* [73], *Bidens pilosa* [74], *Tanacetum cinerariifolium* [75], *Dahlia pinnata* [31]. The progressive interest in the new species is associated with their ability to synthesize commercial biologically active compounds. So, artichoke can synthesize the compounds possessing antioxidant and hepatoprotective activity. The gus-positive (30%) transgenic callus was obtained using *Agrobacterium tumefaciens*-mediated transformation and transgenic *Cynara cardunculus* roots with 45% frequency were obtained using *A. rhizogenes*-mediated transformation [37]. Two strains of *A. rhizogenes* (AR15834 та A4) were used to obtain transgenic roots of *Inula helenium*. The influence of explant type on the transformation process was defined and the transgenic roots from leaf and stem explants of different age were obtained [59]. Transgenic *Centaurea montana* plants were constructed using two *A. tumefaciens* strains, AGL1 and EHA105. However, the efficiency of the transformation was low (1.8% from 990 explants) [73]. In our opinion the great attention may be paid to the usage of *Bidens*...
Since the nineties of the 20th century the great yield of crops and efficiency of agriculture is aimed at increasing phytopathogenic microorganisms and etc. Creation of plants resistant to insects, viral and bacterial can be achieved by creating plants which are resistant to insects, salad plants. Higher yields of these crops are achieved by creating plants which are resistant to insects, viral and bacterial diseases, abiotic stress (drought, soil salinity etc). Creation of plants resistant to insects, phytopathogenic microorganisms and abiotic stress factors is aimed at increasing yield of crops and efficiency of agriculture. Since the ninetieth of the 20th century the great amount of investigations has been dedicated to the development of the system for genetic transformation of sunflower. This oilseed is widely cultivated in the countries of European community, Russian Federation and Argentina. Ukraine is the leader of sunflower growing and the part of Ukrainian production is widely cultivated in the countries of European community, Russian Federation and Argentina. Ukraine is the leader of sunflower growing and the part of Ukrainian production in the global harvest has been increased during the last decade. The interest in sunflower biotechnology is amplified. The optimal conditions of co-cultivation with agrobacteria and selection in the presence of kanamycin after transformation was studied. The efficiency of usage of acetosyringon and some reporter genes (gus, gfp, mgf5) was defined. Transformation efficiency using mature and immature embryos, leaves, varieties or hybrids of H. annuus was compared [83–90]. The content of culture medium was optimized [78]. Transformation using A. rhizogenes was performed to provide better rooting of hybrids [76]. The investigated technological schemes served as a base to obtain crops with valuable properties. Thus, sunflower plants resistant to phytopathogenic fungi Sclerotinia sclerotiorum were obtained using A. tumefaciens-mediated transformation [91]. H. annuus plants resistant to the herbicide Basta at a concentration of 3 l/ha by transferring bar gene were obtained [90]. Ukrainian researchers analyzed the feasibility of using double-stranded RNA suppressor gene of prolin dehydrogenase (based on Arabidopsis ProDH1 gene) to increase the resistance of sunflower plants to stress factors such as water deficiency and salinity. Transgenic H. annuus plants with a high content of free L-proline under the stress conditions were constructed and a reduction of content of this compound during recovery plants after stress was noted. So these transgenic plants possessed the better adaptation ability to stress conditions [93].

Chicory and lettuce are plants grown in different regions (Europe, Asia and America). Some directions of improving the quality of these plants were aimed at obtaining plants resistant to stress factors. As it was reported [45] the chicory plants resistant to salinity were constructed by A. tumefaciens-mediated transformation. Transferring of AtNHX1 gene in chicory genome not only increased the tolerance of plants to salinity, but also reduced the damage of cell membranes induced by a high salt content. Transferring of P5CS gene, which encodes the enzyme delta-pirrrolin 5-carboxyl-synthase involved in the synthesis of proline, made it possible to increase the resistance of lettuce plants to abiotic stress [67]. It is known that abscisic acid acts in the response of plants to the effect of stress factors. Transgenic lettuce plants resistant to drought and cold stress were obtained using ABF3 gene, cloned from Arabidopsis [68]. Viral coat protein genes in two modifications were transferred into Lactuca sativa by A. tumefaciens-mediated transformation and as a result LBVaV virus was not detected in the obtained virus-infected plants.

It was established that CP gene in antisense orientation permit a resistance to phytovirus for one line of plants [71]. Transferring bar gene to L. sativa gave the possibility to obtain plants resistant to the herbicide [65]. The possibility of an efficient transferring Pta gene (Pinellia ternata Agglutinin) into the lettuce plants have been reported, however the researchers didn’t define the resistance of transgenic plants to insects [63].

**Creation of plants resistant to biotic and abiotic stress factors**

Among the Compositae plants there are many species which have economic value and are grown as oil and green cultures. Helianthus annuus is an important oil plant, Lactuca sativa and Cichorium intybus are salad plants. Higher yields of these crops can be achieved by creating plants which are resistant to insects, viral and bacterial diseases, abiotic stress (drought, soil salinity etc). Creation of plants resistant to insects, phytopathogenic microorganisms and abiotic stress factors is aimed at increasing yield of crops and efficiency of agriculture. Since the ninetieth of the 20th century the great amount of investigations has been dedicated to the development of the system for genetic transformation of sunflower. This oilseed is widely cultivated in the countries of European community, Russian Federation and Argentina. Ukraine is the leader of sunflower growing and the part of Ukrainian production in the global harvest has been increased during the last decade. The interest in sunflower biotechnology is amplified. The optimal conditions of co-cultivation with agrobacteria and selection in the presence of kanamycin after transformation was studied. The efficiency of usage of acetosyringon and some reporter genes (gus, gfp, mgf5) was defined. Transformation efficiency using mature and immature embryos, leaves, varieties or hybrids of H. annuus was compared [83–90]. The content of culture medium was optimized [78]. Transformation using A. rhizogenes was performed to provide better rooting of hybrids [76]. The investigated technological schemes served as a base to obtain crops with valuable properties. Thus, sunflower plants resistant to phytopathogenic fungi Sclerotinia sclerotiorum were obtained using A. tumefaciens-mediated transformation [91]. H. annuus plants resistant to the herbicide Basta at a concentration of 3 l/ha by transferring bar gene were obtained [90]. Ukrainian researchers analyzed the feasibility of using double-stranded RNA suppressor gene of prolin dehydrogenase (based on Arabidopsis ProDH1 gene) to increase the resistance of sunflower plants to stress factors such as water deficiency and salinity. Transgenic H. annuus plants with a high content of free L-proline under the stress conditions were constructed and a reduction of content of this compound during recovery plants after stress was noted. So these transgenic plants possessed the better adaptation ability to stress conditions [93].

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**Construction of plants with altered phenotype**

Genetic transformation can lead to a changes in plant phenotype. Such changes were observed at chicory plants which were transformed by A. rhizogenes [41]. Regenerated plants formed flower-bearing stems in the
first year of growth, although these plants are perennial. This peculiarity of genetic transformation and transformation with the usage of specific genes made it possible to isolate new forms of decorative plants. So, transformation of decorative Gerbera jemononii plants with the usage of two genes which are responsible for the color of the flowers – iris-dfr and petunia-f3’5’h have led to the change of flowers pigmentation [35]. Transferring chalcone synthase gene antisense cDNA to gerbera plants provided the suppression of the anthocyanin synthesis and has led to change of pigmentation of some regenerated plants [33].

Although the number of studies concerning the usage of Compositae plants is rather limited, the biotechnological approaches to obtain new forms are promising because of wide usage of decorative plants e.g. Gerbera and significant progress which was made in biotechnological methods to change phenotype of decorative plants [94].

The Compositae family includes numerous species of interest for biotechnological research. However, only some of them are using now in genetic engineering to obtain plants with valuable economic features. There are no investigations in genetic transformation of such plants as Ligularia thomsonii, Xanthium stramarium, Scorzonera undulata, Senecio erucifolius, Tussilago farfara. Some publications dedicated to biotechnological approaches to create transgenic Stevia rebaudiana [95], Bidens pilosa [74], Echinacea purpurea [96, 97] plants is limited. At the same time there is a group of plants that have practical value. Thus, Asteriscus plants synthesize essential oils, which possess antioxidant properties; Vernonia condensata, Scorzonera undulata, Bidens pilosa are the source of antioxidants; some species synthesize flavonoids, phenolic compounds, sesquiterpene lactones; fructose containing compounds with wide spectrum of biological activity, in particular hepatoprotective, prebiotic, antidiabetic, immunomodulatory activity etc.

So, a genetic transformation of new plant species to improve their properties (resistance to stress factors, plants with altered phenotype) as well as creation of plants producing recombinant proteins besides naturally synthesized compounds is a preferable direction of practical usage of plant biotechnological approaches.
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Проаналізовано дані літератури і власних досліджень автора щодо біотехнологічних підходів, які застосовують для генетичної трансформації рослин родини Compositae з використанням Agrobacterium tumefaciens та A. rhizogenes, наведено результати генетичної трансформації рослин низки видів, зокрема їстівних (Cichorium intybus, Lactuca sativa), олійних (Helianthus annuus), декоративних (Gerbera hybrida), лікарських (Bidens pilosa, Artemisia annua, Artemisia vulgaris, Calendula officinalis, Withania somnifera та ін.). Розглянуто деякі напрями генетичної інженерії рослин родини Compositae, зокрема для створення форм, стійкі до хвороб, таких, що не вражаються шкідниками, з новими господарськими ознаками (стійкість до гербицидів, дії абіотичних стресових факторів, зі зміненим фенотипом). Наведено також дані щодо розроблення біотехнології отримання «бородатих» коренів рослин родини Compositae, зокрема Cynara cardunculus, Arnica montana, Cichorium intybus, Artemisia annua.

Ключові слова: Compositae, Agrobacterium tumefaciens, Agrobacterium rhizogenes, "бородаті" корені.