ISSN 0003-6838, Applied Biochemistry and Microbiology, 2016, Vol. 52, No. 2, pp. 121–137. © Pleiades Publishing, Inc., 2016.
Original Russian Text © M.S. Burlakovskiy, V.V. Yemelyanov, L.A. Lutova, 2016, published in Prikladnaya Biokhimiya i Mikrobiologiya, 2016, Vol. 52, No. 2, pp. 149–167.

Plant Based Bioreactors of Recombinant Cytokines (Review)

M. S. Burlakovskiy, V. V. Yemelyanov, and L. A. Lutova

Biology Faculty, St. Petersburg State University, St. Petersburg, 199034 Russia

e-mail: bootika@mail.ru

Received May 12, 2015

Abstract—Cytokines are a family of signaling polypeptides involved in intercellular interactions in the process of the immune response, as well as in the regulation of a number of normal physiological functions. Cytokines are used in medicine for the treatment of cancer, immune disorders, viral infections, and other socially significant diseases, but the extent of their use is limited by the high production cost of the active agent. The development of this area of pharmacology is associated with the success of genetic engineering, which allows the production of significant amounts of protein by transgenic organisms. The review discusses the latest advances in the production of various cytokines with the use of genetically modified plants.

Keywords: plant-based production systems, genetic transformation methods, cytokines, erythropoietin, interleukins, interferons, growth factors

DOI: 10.1134/S0003683816020034

INTRODUCTION

The genetic engineering of plants as a trend in biotechnology was formed after the development of cellular and molecular biology, microbiology, and genetics. The main stages of DNA modification in living organisms are the search for and decoding of regulatory regions of the genome (promoters, terminators) and sites encoding the amino acid sequences of the proteins; their excision and fusion in the required combinations; and introduction into the cell genome. Biotechnological methods make it possible to overcome species barriers and perform recombination outside the organism with high accuracy. In the simplest case, genetic modification leads to the appearance of foreign proteins in the cell or changes in the synthesis level of one of the cell proteins.

One of the first practical uses of genetic engineering was the production of bioreactor organisms that synthesize large amounts of foreign proteins, the production of which from natural sources is unprofitable and associated with the risk of disease transmission or ethical problems. Such produced and purified recombinant proteins can be used for functional analysis and structural and biophysical studies, as well as in pharmacology. For example, mammalian insulin was synthesized in *Escherichia coli* in 1977 [1]. After 5 years, the use of this hormone, which is produced by bacteria, was approved by FDA (US Food and Drug Administration), giving rise to the use of recombinant proteins in the medicine. Expression systems based on bacterial, insect, and mammalian cell cultures [2, 3], yeast [4], and plants [5] are currently used for commercial protein synthesis. Such areas as the production of the heterologous protein in the milk of transgenic animals [6] and the cell-free protein synthesis system are also being developed [7].

Each system is characterized by its own advantages and disadvantages associated with both the biological properties of the object and with the economic requirements for its use. Although the genetic code is universal, different organisms have dissimilar mechanisms of RNA processing and post-translational processing of the protein. Thus, prokaryotic cell splicing of eukaryotic genes (intron excision) does not occur, which requires a further modification of the heterologous gene prior to transformation. Differences in such processes as the folding of the recombinant protein, its glycosylation, phosphorylation, and the formation of disulfide bonds may decrease its biological activity until a complete loss of function or cause an allergic reaction as the result of its therapeutic use. Foreign proteins can be toxic to the producer [8, 9]. It is generally accepted that a closer congeniality of the producer and donor provides the best results. Thus, expression systems in mammalian cells are the most successful for the production of proteins for medical purposes. However, they are not suitable for the production of proteins with high signaling activity, in particular, hormones, and lose out to alternative systems in terms of production costs and biosafety. A universal system of gene synthesis of proteins does not currently exist, and it is often impossible to predict which of the existing systems will be better for the industrial production of one or another protein [2, 9].

Plant-based production systems. Among the currently available transgenic organisms, plants are of the

utmost economic importance due to the possibility of their large-scale agricultural use. Molecular breeding is an area of biotechnology that solves the classic problems of agriculture by traditional genetic modification of plant varieties for their adaptation to intensive farming methods. The genetic transformation of plants was developed in the 1980s [10]. To date, transgenic plants resistant to herbicides, pests, and pathogens, as well as plants with balanced, modified compositions of amino acids, fatty acids, and secondary metabolites, have been produced and implemented. These advances allow us to improve agricultural productivity and maintain global food production constantly to meet the requirements of the growing world population [11–13]. Intensive research in this area has led to the introduction of simple and well-characterized methods of plant transformation that have served as the basis for the use of plants as producers of pharmaceutical proteins. Human growth hormone [14] and IgG1 (**6D4**) antibody fragment [15] were produced from transgenic tobacco (*Nicotiana tabacum* L.) and sunflower plants (*Helianthus annuus* L.) shortly after the technology of plant transformation appeared. In 1997, the recombinant avidin, which is suitable for commercial use, was isolated from transgenic maize (*Zea mays* L.), confirming the possibility of producing recombinant proteins on a large scale [16]. Since then, numerous antibodies, vaccines, cytokines, and other proteins were produced by plant expression systems with the use of both mature plants and cell cultures [13, 17].

As compared with other expression systems (bacteria, microorganisms, and animal cell cultures), plants have a number of features and advantages. First, they provide high infection security from plants to humans, since they are free of pathogens and animal viruses and can be grown without the use of materials of animal origin. For biomass growth they require only light, water, carbon dioxide, and minerals, which simplifies and reduces the cost of the production. In comparison with microorganisms, glycosylation systems and other post-translational modifications of proteins in plants and mammals are close [8, 18, 19], which makes it possible to obtain complex glycoproteins with preservation of their biological activity and stability [20, 21]. For plants, there are by now a variety of relatively simple and well-established transformation techniques. The heterologous gene can be integrated into the nuclear or chloroplast genome or into a vector for a temporary (transient) expression. Transgenic plants can be cultivated both in vitro in cell suspension culture or in a callus tissue and in vivo in the whole organism; agricultural cultivation technology makes it possible to achieve a minimal price per biomass unit among transgenic expression systems [22].

The use of different promoters allows the accumulation of the target protein in certain organs of the plant—leaves, roots and seeds. This facilitates the collection of raw material. The addition of the signal of subcellular localization in the vacuole or apoplast to the target protein enables long-term storage and transport of the product without conservation or freezing. Finally, for some proteins used for medical purposes that have an effect when administered orally, there is the possibility of avoiding procedures of isolation, purification, and injection through the use of plants as edible immunomodulators and vaccines. This eliminates the most complex and expensive production steps, which require skilled personnel. These actions can dramatically reduce the cost of the accumulated recombinant protein in plants and simplify their use [17, 22, 23]. Most current transgenic plant-based production systems were created from a soil bacterium, the plant pathogen *Agrobacterium tumefaciens,* and related bacteria with natural transformation mechanisms. The Ti-plasmid of *A. tumefaciens* carries a set of genes responsible for the transport of a fragment of the same plasmid into a plant cell and its integration into the genome. A DNA fragment of Ti-plasmid inserted into the nuclear genome of the host cell was designated as **T-DNA**. Any genetic construct can be inserted into in the T-DNA and transferred into the plant genome by molecular biology techniques [24]. The method of *A. tumefaciens* use (Agrobacterium-mediated transformation) is convenient and simple; it has a high probability and accuracy of the integration of the heterologous gene and the ability to work with fairly long (up to 150 kb) DNA fragments. This method is used for the transformation of both dicotyledonous and monocotyledonous plants with certain limitations, and it is the de facto standard method in the production of transgenic plants [22, 25, 26].

An alternative to Agrobacterium-mediated transformation is the bioballistic method, in which cells are bombarded with microscopic fragments of an inert metal (gold or tungsten) coated with vector DNA [27]. This is one of the few universal genetic engineering techniques that allows DNA to penetrate via the plant cell wall. The advantages of the bioballistic method include independence from the plant species, the ability to transform the nuclear genome and the genome of the chloroplasts or mitochondria, and the absence of flanking sequences of insertion specific to Agrobacterium vectors. The disadvantages of the method are the reduced frequency of the transformation and multiplicity of the insertion [26].

The advantage of the transformation of the nuclear genome is the stable inheritance of the transgene insertion in the number of generations, which makes it possible to obtain an unlimited number of descendants from a single transformant. The main disadvantage of the nuclear transformation is the low transgene expression levels, whereby the content of the recombinant protein often does not exceed 1% of soluble cellular proteins and may decrease depending on the place of the insertion of the heterologous gene and the activation of the silencing process. The possibility of free plant propagation is also associated with the risk of uncontrolled spread of the transgene in nature and

in crossing with other varieties and wild relatives of cultivated plants [5, 23].

An alternative to nuclear transformation is the introduction of the transgene into the chloroplast genome. Each mesophyll cell has about a hundred of these organelles with their own expression system similar to prokaryotic. Transplastomic plants are not exposed to the silencing effect; they have consistently high expression and recombinant protein content, which can reach up to 70% of the cell protein [28]. The absence of plastid genome inheritance from pollen eliminates the threat of uncontrolled spread of the transgene during plant cultivation in the field. One promoter may control the synthesis of several proteins due to operon organization of the genetic material of the chloroplast [13]. The synthesis of heterologous proteins in plastids may decrease their toxic effect on the plant cell. However, a disadvantage of this expression system is that it is impossible to control the direction of recombinant protein in the endoplasmic reticulum, and therefore a conduction of series of post-translational modifications occurs [22]. The production of transplastomic plants is a more complex and unreliable procedure than Agrobacterium-mediated transformation [17].

In plant cells, in addition to the chloroplasts, there is another type of organelle with its own genetic material—the mitochondria. Successful attempts at a transformation of their genome were recently carried out. Heterologous constructs were introduced into the mitochondria of the unicellular alga *Chlamydomonas reinhardtii* Dangeard by the bioballistic method [29] and into the mitochondria of *Arabidopsis thaliana* (L.) Heynh. with transmembrane protein complexes [30]. The advantage of this technology, as in the case of chloroplasts, is the large number of mitochondria in the cells and the high transcriptional activity of their genome, which can provide significant levels of recombinant gene expression. However, current methods of mitochondrial transformation remain experimental and do not have commercial value.

Another promising method is transient expression technology, which does not result in the emergence of a heterologous gene into the generative organs of the plant. Thus, the agroinfiltration method includes treating leaf tissue of tobacco with an *Agrobacterium* suspension in the presence of a factor that damages cell membranes, such as pressure or vacuum, which leads to a massive infection of cells, the appearance of multiple T-DNA copies in cells, and the production of a large amount of recombinant protein in a short time [31]. The delivery method of the genetic construct by modified plant viruses, the propagation of which gives rise to multiple copies of the heterologous gene and intensive production of protein, is also common [32]. There is also a hybrid technology in which the viral vector is deprived of the proteins responsible for penetration through the cell membrane and is introduced into plant cells as part of the T-DNA of *A. tumefaciens*, thus increasing the yield of the target protein [33]. Transient expression vectors are not inherited during the sexual reproduction of plants, eliminating the threat of uncontrolled spread of the transgene. Transient expression allows plant growth under controlled conditions. Among its disadvantages are the limited amounts of recombinant protein resulting from a single act of transformation and the need for constant production of the vector system in large quantities. Transient expression does not require the regeneration of a whole plant, and it can be used to produce recombinant proteins within a few days. This system may be used to quickly target the production of therapeutic proteins, especially vaccines, in a limited time [34, 35].

It is not necessary to use a multicelled plant organism for the production of plant biomass. At the present time, unicellular algae, in particular, *Chlamydomonas reinhardtii,* are used as producers [13]. The technology of plant biomass production in the form of culture, the so-called "hairy roots" (the result of transformation with *Agrobacterium rhizogenes*), is also promising [36].

The suspension cell culture of higher plants is a widely used biotechnology model for the commercial production of recombinant proteins. The use of bioreactors has such advantages as the availability of a well-proven technology and scalability. Isolation from the environment provides safety from infection by pathogens of mammals and eliminates the possibility of spread of the transgene in the environment, which is ideally suited for the production of highly purified pharmaceutical proteins and facilitates the registration of the product. The high growth rate of the cell culture and the lack of necessity for full regeneration of plants provide a sufficient amount of recombinant protein in a short time. In the suspension culture conditions, cells with thin cell walls are able to secrete the recombinant protein in an environment that facilitates its isolation and purification. This also makes it possible to achieve a higher uniformity of recombinant protein properties when compared to those obtained in the whole plant, which is associated both with the strictly controlled conditions in a bioreactor and shorter-term contact of the protein with various intracellular enzymes [19, 37].

At the present time, it has been shown that transgenic plants are capable of synthesizing a variety of functional pharmaceutical proteins of different families, many of which undergo clinical trials, and close to commercial use. Thus, Protalix (Israel) produces biopharmaceuticals registered under the name Elelyso and approved for the treatment of Gaucher disease type 1 (cerebroside lipidosis, lysosomal storage diseases caused by lack of the enzyme called glucocerebrosidase and the accumulation of glucocerebroside in many tissues of the body). The active ingredient is glucocerebrosidase, which is produced in the suspension culture of carrot cells (*Daucus carota* L.). The recom-

Table 1. Cytokine family (http://www.kegg.jp/kegg-bin/get_htext?htext=ko04052.keg&query=cytokines)

Class	Cytokines family	Cytokine
Class I Cytokines (hematopoietins)	Interleukin 2 family	IL-2, 4, 7, 9, 13, 15, 21
	Interleukin3 family	Granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, 5
	Interleukin 6 family	Oncostatin M (OSM), leukemia inhibitory factor (LIF), granulocyte colony stimulating factor (G-CSF), cardiotrophin 1, leptin
	One subunit hematopoietins	Erythropoietin (EPO), prolactin
Class II Cytokines	Type I interferons	Interferons alpha, beta, omega 1, kappa, tau 1
	Type II interferons	Interferons gamma
	Type III interferons	Interferon lambda, aka IL 28/29
	IL-10 family	IL-10, 19/20/24, 22, 26
Platelet derived growth factor family (PDGF)		IGF-1, IGF-2
Tumor necrosis factor family (TNF)		TNFA, TNFB, TNFC, TNFSF4...
IL-1 family		IL-1A, 1B, 1RN, 18, F5, F6, F7, F8, F9, F10
IL-17 family		IL-17, 17B, 17C, 17D, 17E, 17F
Tumor growth factor family (TGF-beta)	9 families	TGF-beta 1, 2, 3, Bone morphogenetic proteins (BMP)
Chemokines	CC family	CCL2, CCL3, CCL4...
	CXC family	CXCL1, CXCL4, IL-8...
	CX3C family	CX3CL1
	C family	XCL

binant protein accumulates in the vacuoles of cells via the signal of intracellular transport from the tobacco chitinase [38]. The development of a recombinant vaccine against the SARS virus H1N1 (Medicago, Canada), which was based on a commission from Defense Advanced Research Agency (DARPA, United States), is of considerable interest. The protein is produced within a day in tobacco plants exposed to agroinfiltration, which allows for quick production in the case of an epidemic. A vaccine, the clinical trials of which are currently being finalized, is one of the most promising pharmaceutical proteins of plant origin [39]. The production of the chimeric antibodies to the Ebola virus, which have demonstrated clinical efficacy in the transgenic tobacco plants, also generated interest [40]. Overall, the production of recombinant proteins using transgenic plants is taking place in about 20 companies, including Biolex (United States), Dow AgroSciences (United States), Icon Genetics (Germany), Large Scale Biology (United States), Medicago (Canada), Meristem Therapeutics (France), Novoplant (Germany), ORF Genetics (Iceland), Planet Biotechnology (United States), ProdiGene (United States), Protalix Biotherapeutics (Israel), and others [13, 17].

Cytokines and their use in medicine and veterinary science. Blood proteins are of particular interest for the pharmaceutical industry, since they play a key role in metabolisms. Blood proteins may be divided into two basic types: one (I) includes proteins related to the circulating cells and the other (II) includes hormones, cytokines, enzymes, protease inhibitors, coagulation factors and lipid, vitamins, and ions transfer proteins. Plasma proteins are used for the treatment of evoked or congenital diseases, including various forms of hemophilia and other hematologic diseases and immunological disorders [41].

Cytokines are a group of endogenous protein mediators of intercellular interactions involved in the regulation of proliferation, differentiation, and cell motility (Table 1). Cytokines are important components of the immune system, but they are also involved in embryogenesis and affect the hematopoietic system and nervous cells. More than 250 individual compounds having common properties are currently referred to cytokine system. Cytokines are polypeptides with molecular weights from 5 to 50 kDa, often glycosylated, that may consist of one, two, three, or more identical or different subunits. They are often called hormones of the immune system based on their char-

acteristics. Cytokines are synthesized and perceived by cells of different origin; they have effect in extremely low concentrations, interacting with highly specific cell receptors. They may affect the whole body after penetration into the blood stream (endocrine), nearby cells, for example, at the site of inflammation (paracrine), and the cells synthesizing this cytokine (autocrine). Some researchers separate cytokines into a new separate regulation system existing along with the nervous and endocrine systems for the maintenance of homeostasis [42]. The cytokine network is characterized by interconnectivity, signaling redundancy, and often the opposite role of the same cytokine at different stages of the development process [43].

The role of cytokines in the regulation of body functions can be divided into six major components [42]:

(1) regulation of developmental processes, including fertilization, embryogenesis, anlage and development, in particular the immune system;

(2) maintenance and regulation of certain normal physiological functions, particularly hematopoiesis;

(3) participation in all types of protective reactions of the organism, maintenance of the homeostasis under the influence of internal and external damaging factors;

(4) control of the development of immunopathological processes (allergic, autoimmune and inflammatory reactions);

(5) involvement in the regulation of tumor cell transformation and in the development and metastasis of tumors;

(6) regulation of regeneration processes.

Cytokine classification may be done based on both biochemical and physiological properties, based on the types of receptor binding cytokines [39, 42, 44]. At the moment the most common classification takes into account all of these parameters. It should be noted that, due to the variety of cytokines and their long study, some molecules were independently characterized by different groups of researchers, for example, interleukins 19, 20, and 24 represent the same compound. Many established names also belong to the early period of cytokine research, when the scale of this group of proteins was not known. The term "interleukins" was originally used to designate those cytokines for which leukocytes were the main target cells. The vast majority of them are produced by T-helper cells. Lymphokines are cytokines synthesized by lymphocytes; monokines are synthesized exclusively by monocytes. Interferons are cytokines involved in antiviral response. Colony-stimulating factors support the growth of cells of different pathways of hematopoiesis. Chemokines mediate chemoattraction (chemotaxis) between cells. Tumor necrosis factors induce cell apoptosis, etc.

Cytokines regulate some important functions of the body and play a key role in the immune response to pathogens of any nature, which explains their use in pharmaceutics. Today, several proteins of this family are widely used worldwide as a drug (Table 2). The greatest success of cytokine therapy was achieved with the stimulation of hematopoiesis in bone marrow and the treatment of various infectious diseases and tumors, including in combination with other drugs.

In Russia and abroad, several recombinant cytokine drugs were designed and registered, including erythropoietin, alpha and gamma interferons, colony-stimulating factors, interleukins and their antagonists, and some others. Microbial systems (yeast or *E. coli* suspension) with a productivity of more than 5 g of interferon per 1 L of cell culture are traditionally used to produce the used recombinant cytokines [4, 45]. At present, there are various recombinant cytokines on the market approved for clinical use in the treatment of neutropenia, leukemia, and chronic hepatitis B and C [44]. The use of cytokines as adjuvants for enhancing the immune response during vaccination is also promising [46, 47]. The list of adjuvants in 2012 included nine cytokines, none of which was licensed for medical use [48]. However, the limited use of cytokines is associated with numerous side effects, including, in particular, an increased probability of autoimmune diseases and flu-like syndrome [49].

A wider use of cytokines is observed in the market for veterinary drugs. In 2014 Probioteh (Republic of Belarus) developed diluent adjuvant for live vaccines for cattle based ion recombinant gamma-interferon (**IFN**-γ) (http://www.probiotex.ru). *Pichia pastoris* yeast strains, synthesizing secretory and intracellular cytokines: bovine and chicken IFN-γ [50, 51], chimeric proteins consisting of serum albumin crosslinked with interleukin (**IL**)-2 or human IFN-α16, [52, 53] etc, have been created.

The use of cytokine drugs, particularly IFN in farm animal production, has considerable advantages over traditional antibiotics and chemotherapeutic agents. IFN-γ is used for the treatment of viral, oncological, and several bacterial diseases. It was shown that IFN-γ plays an important role in the immune response to *Mycobacterium tuberculosis bovis* [54, 55], and it is an antitumor agent activating antineoplastic macrophage function [56]. Since viral leukemia is a widespread disease in cattle, the need for such drugs in veterinary medicine is beyond any doubt.

Cytokines of class I (hematopoietins). Hematopoietins are one of the first recombinant cytokine drugs included in routine clinical practice. The reason for this is the stimulation of the proliferation and differentiation of various hematopoietic lineages in bone marrow and the well-studied consequences of therapy, as well as the broad scope of application. Such proteins as erythropoietin (**EPO**) and granulocyte-macrophage colony stimulating factor (**GM-CSF**) consist of a single polypeptide chain, which facilitates their synthesis in transgenic producer. EPO is highly glycosylated cytokine with a size of 30–38 kDa that acts as a pep-

Table 2. Cytokines approved for clinical use [42, 44]

Cytokine	Disease	Examples of drugs (Producer)
Granulocyte colony stimulating factor	Neutropenia	Neupogen/Filgrastim (Amgen, United States), Lenograstim/Granocyte (Chugai Pharmaceuticals, Japan), Granogen (LLC Pharmapark, Russia)
Granulocyte-macrophage colony stimulating factor	Leukemia	Leukine/Sargramostin (Immunex, United States), Molgramostim/Leucomax (Schering Plough, United States)
Interferon-α	Viral infections, cancer	Intron A (Schering Plough, United States), Roferon A (Hoffman La Roche, Switzerland), Infergen (Three Rivers Pharmaceuticals, United States), Alferon N (HEMISPHERx Biopharma, United States), Pegasys (Genentech/Hoffman La Roche, Switzerland), Pegintron/Sylatron (Merck, United States), Multiferon Altevir (LLC Pharmapark, Russia), Alpharona (LLC Pharmaclon, Russia), Viferon (LLC Feron, Russia), Lifeferon (Vectormedica, Russia), Genferon (Biocad, Russia)
Interferon -β	Multiple Sclerosis	Betaseron (Bayer, Germany), Avonex (Biogen Idec, United States), Rebif (Merck Serono, Germany), CinnoVex (CinnaGen, Iran), Interferon-beta-1b (Biocad, Russia), Interferon-beta-1b (Generium, Russia)
Interferon -γ	Osteoporosis Chronic granulomatosis	Ingaron (LLC SPE Pharmaclon, Russia), Actimmune (Horizon Pharma, United Kingdom), Imukine (Boehringer ingelheim, Germany)
Erythropoietin	Anemia of different nature	Eprex (Janssen-Cilag, Switzerland), Epogen (Amgen, United States), Epocrin (Federal Unitary Enterprise State Research Institute of highly pure biopreparations, Russia), Epostim (Pharmapark, Russia)
Interleukin-2	Cancer	Aldesleukin (Novartis, Switzerland), Proleukin (Prometheus Laboratories, United States), Interking (Shenzhen Neptunus Interlng-H, China), Roncoleukin (Biotech, Russia)
Interleukin-11	Thrombocytopenia	Oprelvekin/Neumega (Pfizer, United States)
Bone morphogenetic proteins (BMP)	Fractures, mouth operations	Infuse BMP-2 (Medtronic, Ireland), OP-1 BMP-7 (Stryker Biotech, United States)
Stem cell growth factor (SCF)	Cancer, bone marrow transplantation	Ancestim (Amgen, United States)

tide hormone in regulating the differentiation of progenitor cells of mature erythrocytes (erythropoiesis). In adults, EPO is mainly produced in the interstitial cells of the peritubular capillaries of the kidney. Up to 10–15% of EPO is synthesized by hepatocytes and epithelial cells surrounding the central veins of the liver. The *Epo* gene is expressed at minimal levels in other organs and tissues (brain, placenta, spleen, and lungs). The signal for EPO synthesis is a decrease in the partial oxygen pressure in the tissues and the subsequent development of hypoxia, which occurs as a result of reduced transport of oxygen resulting from the decreased number of circulated erythrocytes. Under normal physiological conditions, EPO is constantly present in the blood plasma in low concentrations in the range of 10–15 mIU/mL. Recombinant EPO is widely used at present for the treatment of anemia of different natures, because it provides the opportunity to restore proper hematopoiesis and to compensate for the anemia without the transfusion of donated blood [42]. In 2003, global demand for EPO was estimated at 2 kg [39], and the cost of recombinant EPO reached $4000/mg [30].

Human EPO is a glycosylated polypeptide comprising three carbohydrate chains bound to the amide groups of Asn-24, -38 and -83 residues and one chain with the OH– group of Ser-126 residue. For the full manifestation of protein activity and stability in the bloodstream, the N-glycan residue of sialic acid, which is a characteristic feature of the human glycoprotein, should be present [57, 58].

EPO was one of the first recombinant cytokines produced in plant cells [57, tab. 3]. The human EPO gene was isolated from cDNA and provided with a 35S promoter of cauliflower mosaic virus (**CaMV**). The signal peptide in the N-terminal region caused the extracellular localization of the recombinant protein. Since EPO had a molecular weight of 30 kDa, its free migration through the cell wall into the cell cultures was difficult. Therefore, EPO secretion occurred in tobacco protoplasts. Recombinant EPO was synthesized in the plant cells and was also glycosylated, like native EPO, but the composition and the length of the polysaccharide chains were different. Unfortunately, the recombinant protein yield was low (0.0026% of the total soluble protein). The protein was biologically active in vitro, but in vivo activity was not detected. This can be explained by differences in the glycosylation, which is known to affect the stability of the protein in blood [57]. High levels of transient gene expression of the *Epo* gene was achieved in protoplasts of moss *Physcomitrella patens* (Hedw.) Bruch & Schimp. wild-type and Δ-*Fuc-T* Δ-*xyl-T* mutant lines (up to 0.250 mg/g dry weight) [58]. Expression of the *RhEpo* gene was observed in the whole tobacco and Arabidopsis plants. All transgenic lines had a high level of transcription, but the tobacco plants had abnormal morphology with delayed vegetative growth and male sterility [59].

Granulocyte-macrophage colony stimulating factor (**GM-CSF**) is a major growth factor for neutrophilic granulocytes and monocytes that stimulates the proliferation and differentiation of intermediate and late progenitor cells into neutrophils, eosinophils, and monocytes. The therapeutic use of GM-CSF effectively restores the number of granulocytes in peripheral blood (which is reduced, in particular, as a result of high-dose cancer chemotherapy) and prevents the development of infectious complications. GM-CSF can be used in clinical practice for the treatment of neonatal neutropenia and in haemato-oncology for the treatment of infectious diseases [42]. GM-CSF is a protein with a molecular mass of 22 kDa that is secreted primarily by macrophages and T-cells participating in the immune and inflammatory cascades. GM-CSF does not exhibit structural variability, and patients tolerate it well; therefore, it is a candidate for use as a pharmacological agent [60].

Initially, recombinant human GM-CSF was synthesized in tobacco [60, 61] and rice (*Oryza sativa* L.) suspension cell cultures [62–64]. One of the most interesting approaches to increase the yield of protein includes the introduction of mineral salts or bovine serum albumin into the nutrient medium, which stabilized the secreted cytokine [60]. A significant (up to fourfold) increase in the yield was also achieved by adding a gelatin to the medium (up to 5% by weight). However, in cultures older than 4 days, the gelatin inhibited cell growth, probably by activating proteolytic enzymes [61]. In the rice suspension culture cells, the yield of the desired protein was 129 mg/L of the culture due to the use of amylase promoter Ramy3D in the absence of sugar, which is necessary for promoter activity [62]. The system was subsequently optimized by the following three approaches: (I) amylase gene silencing (amylase is the dominant protein in rice cells); (II) rice cysteine protease gene silencing; and (III) co-expression of the cytokine with protease inhibitor [64]. Each of these strategies increased the yield of recombinant GM-CSF by at least two times. Mouse GM-CSF (mGM-CSF) was successfully obtained in rice suspension culture cells with a yield of 24.6 mg/L medium [65]. The same glycoprotein was expressed in tobacco leaves with a yield of 19 μg/g fresh leaves [66]. The biological activity of recombinant human and mouse GM-CSF obtained in rice suspension cell culture was confirmed with GM-CSF-dependent TF1 cells and mouse NFS-60 myeloid leukemia cells, respectively, [21, 65]. Full-grown plants were used for the production of GM-CSF. The maximum yield of recombinant protein reached approximately 0.02% [67] in the leaves of transgenic sugarcane (*Saccharum officinarum*) and approximately 0.22% of the total soluble protein in tobacco leaves [66]. In the seeds of transgenic plants, recombinant GM-CSF was up to 1.3% of the total protein in rice [68], and up to 0.03% of the total soluble protein in tobacco [69]. The highest amount of protein (up to 2%) was synthesized by using the potato virus X (PVX) with a modified protein of the envelope as the vector [70]. The protein was also synthesized in such expression systems as rice and tobacco seeds with the rice glutelin GT1 and GT3 promoters. In most cases, the biological activity of recombinant GM-CSF was confirmed in vitro and in vivo by experiments with mice [71] and hamsters [72].

IL-2 plays the role of a growth factor for all subpopulations of T-lymphocytes and also activated B-lymphocytes, thus being an important stimulator of specific immune response, and it also has antitumor activity. IL-4 affects primarily B-lymphocytes and inhibits the synthesis of some proinflammatory cytokines (tumor necrosis factor, **TNF**, IL-1 and IL-6). IL-2 has strong adjuvant properties, e.g., the ability to enhance the effect of vaccination when administered simultaneously with the antigen [73]. The first report on the biosynthesis of recombinant IL-2 and IL-4 in tobacco cell suspension culture was published in 1998 [74]. Tobacco cells were transformed with a construct carrying the *hIL-2* sequence of cDNA and synthesized up to 0.38 mg human IL-2/g callus tissue or 0.08 μg/mL of the liquid cell culture. It was assumed that the presence of a signal peptide in the N-terminal region would provide an extracellular localization of recombinant proteins. However, the effectiveness of the secretion was insignificant, and the majority of the produced cytokines remained in the cells. Only proteins isolated from cells had the ability to maintain in vitro proliferation of IL-2-dependent murine cell line

CTLL-2. In the study [75], the expression of *hIL-2* in potato (*Solanum tuberosum*) tubers was performed under the control of tissue-specific promoter. The activity of the recombinant protein was 115U/g of plant tissue. In a later study [76], different lines of transgenic tobacco plants synthesizing both individual mature IL-2, and IL-2 in combination with two different protease inhibitors were produced. The recombinant protein contained six histidine residues (**Tag**) at the N-terminal region, and it was ligated to the C-terminal localization signal in the endoplasmic reticulum. The yield of protein was 1.8–9.5 μg IL-2/g fresh leaf and 0.4–3.5 μg IL-2, which is crosslinked to protease inhibitor/g fresh leaf. Both IL-2 forms synthesized in CTLL-2 cell culture had biological activity, the expression of which was not impaired by the presence of Tag, c-Myc, or proteinase inhibitors to the molecule. Recombinant IL-2, which is crosslinked to Tag or c-Myc and bears a localization signal to the endoplasmic reticulum or the chloroplast, was synthesized in tobacco plants [77]. The protein yield was not measured, but the purified IL-2 exhibited activity on CD4 + T-cells from mouse spleen.

Plants producing interleukins of different families with biological activities were obtained by *Agrobacterium* transformation: human IL-4 in tobacco [74] and potato suspension cell culture [78], anti-inflammatory human cytokine IL-10 in whole tobacco plants [79] and the suspension cell culture [80, 81], as well as in *A. thaliana* plants [82]. This suggests that the post-translational modification of proteins in plants allows these recombinant cytokines function in mammals. The mature form of IL-1b, IL-7, IL-10, mIL-4, and mIL-18 with the signal of intracellular transport into the endoplasmic reticulum attached to the C-terminal residue were synthesized in the endosperm of transgenic rice plants [83]. The activity of these recombinant interleukin in mammalian cells was tested. Transplastomic plants were also used to produce cytokines [84].

It should be noted that the incorporation of the poly-histidine Tag sequence can improve protein synthesis and facilitate its purification [85] but may complicate its use as a drug if these extraneous residues affect the biological activity or cannot be effectively removed.

Such class I cytokines as the cardiotrophin 1 [86], IL-12 [87–89], IL-13 [90], and IL-18 [91] were synthesized in the transgenic plants. Interestingly, IL-12, which consists of two monomers, was synthesized with a yield of more than 5% of the total soluble protein after double agroinfiltration of *Nicotiana benthamiana* Domin leaves with two *Agrobacterium* strains, each of which individually encoded one subunit [92]. IL-12 produced in transgenic tomatoes had biological activity comparable to the activity of the substance produced in *E. coli* [88]. Cardiotrophin 1 was expressed in the transplastomic tobacco plants with a yield of about 5% [86].

Class II cytokines. Interleukin-10 (**IL-10**) is a cytokine that plays an important role in numerous inflammatory and immunoregulatory reactions [42]. It was successfully synthesized in rice seeds under the regulation of Glub-1 promoter. The target protein contained a signal peptide for localization in the endosperm of rice seeds (Table 3). It was found that the glycoside group of the synthesized product and the native IL-10 were indistinguishable. The yield of purified target protein was 50 mg/g of seed. Recombinant IL-10 showed biological activity on dendritic mouse bone marrow cells, [93]. IL-10 was expressed in tobacco tissues (yield 37.0 mg/g fresh leaves) [94].

IFN are glycoproteins, a potent stimulator of the immune system against pathogens and tumors of different nature. Recombinant IFN of bacterial origin are usually used for the treatment of Kaposi's sarcoma, myeloid leukemia, and hepatitis A and C. [95, 96]. IFN-γ belongs to class II interferons and is responsible for the regulation of antiviral and antitumor immune response [42]. IFN is often expressed in plant-based systems. The first reports on the production of human interferon were in the early 90s. [97]. Human IFN was expressed in transplastomic tobacco plants, which determined a significant level of its synthesis (20% of the total soluble protein) [98]. Not only human interferons but bovine [99], hen [100], and salmon interferons [101] were successfully obtained in plant-based production systems. IFN-γ was synthesized in rice suspension cell culture with the use of the constitutive maize ubiquitin promoter, an inducible rice promoter αAmy3/Ramy3D. The signal peptide of the alpha-amylase was used to ensure the secretion of the recombinant IFN-γ in culture medium in both cases. The recombinant protein had biological activity on an A549 human cell line infected with dengue virus (**DENV**). The highest yield of the desired product under the control of the ubiquitin promoter was 12 ng/mL of culture medium (699.79 ng/g fresh cells) and 17.4 ng/mL (131.6 ng/g fresh weight of cells) of αAmy3 / Ramy3D promoter [102]. Transgenic constructs for the coexpression of *Mycobacterium tuberculosis* antigens and IFN-γ in plants for the production of an "edible vaccine" were obtained [103].

The preparation of recombinant IFN-2b (Locteron®) was synthesized in duckweed plant (*Lemna minor* L.), which is used for the large-scale production of monoclonal antibodies and interferon [38, 104]. This drug was subsequently licensed by OctoPlus NV (Holland). After clinical trials IFN-2b became one of the first recombinant therapeutic cytokines to reach commercial use (http://www.octoplus.nl/files/4713/4676/5370/OctoPlus_announces_publication_of_positive_Locteron_interim_Phase_IIb_data.pdf).

Tumor necrosis factor (**TNF-α**), which belongs to the TNF family, was synthesized in the potato with the use of two types of expression vectors under the control of CaMV 35S promoter. The transformation vec-

Table 3. Recombinant cytokines expressed in plants [39]

Cytokine	Plant	Growing method	Transformation method	Content	Source
Erythropoietin	*Nicotiana tabacum*	Suspension	Agrobacterium-mediated transformation	0.0026% total soluble protein	[57]
Erythropoietin	*Physcomitrella patens*	Suspension	Polyethylene glycol-mediated transformation of protoplasts	0.05 mg/g	[58]
Erythropoietin	*Physcomitrella patens*	Suspension	Polyethylene glycol-mediated transformation of protoplasts	0.123 mg/L	[58]
Erythropoietin	Tobacco	Entire plants	Agrobacterium-mediated transformation	0.231 mg/g total soluble protein	[30]
Erythropoietin	*Nicotiana benthamiana*	Transient expression	Agroinfiltration	52 μg/g	[123]
Human granulocyte colony stimulating factor (G-CSF)	*Lactuca sativa*	Transplastomic plants	Bioballistic	No data	[124]
Human granulocyte-macrophage colony stimulating factor (GM-CSF)	Tobacco	Suspension	Agrobacterium-mediated transformation	Maximum 0.25 mg/L	[60]
GM-CSF	Tobacco	Suspension	Agrobacterium-mediated transformation	Maximum 0.78 mg/L	[61]
GM-CSF	*Oryza sativa*	Suspension	Bioballistic	0.129 g/L	[62]
GM-CSF	Tobacco	Suspension	Agrobacterium-mediated transformation	0.105 mg/L	[125]
GM-CSF	*Saccharum officinarum*	Entire plants	Bioballistic	0.02% total soluble protein	[67]
GM-CSF	*Solanum lycopersicum*	Suspension	Agrobacterium-mediated transformation	45 μg/L	[126]
GM-CSF	*Oryza sativa*	Suspension	Bioballistic	0.28 g/L	[63]
GM-CSF	*Oryza sativa*	Suspension	Bioballistic	0.29 g/L	[63]
GM-CSF	*Oryza sativa*	Suspension	Bioballistic	0.25 g/L	[64]
GM-CSF	*Oryza sativa*	Entire plants	Agrobacterium-mediated transformation	1.3% total soluble protein	[68]
GM-CSF	Tobacco	Entire plants	Agrobacterium-mediated transformation	Maximum 0.03% total soluble protein	[69]
GM-CSF	*Oryza sativa*	Mature plants	Agrobacterium-mediated transformation	Maximum 0.014 μg/seed	[71]
GM-CSF	*N. benthamiana*	Transient expression	Viral transformation	Maximum 2% total soluble protein	[70]
Mouse GM-CSF	*Oryza sativa*	Suspension	Bioballistic	24.6 mg/L	[126]
Mouse GM-CSF	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	19.9 μg/g	[66]
IL-2	*Nicotiana tabacum*	Suspension	Agrobacterium-mediated transformation	80 μg/L	[74]

Table 3. (Contd.)

Cytokine	Plant	Growing method	Transformation method	Content	Source
IL-2	*Solanum tuberosum*	Entire plants	Agrobacterium-mediated transformation	1.15–105 U/g	[75]
IL-2	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	9.5 μg/g	[76]
IL-2	*Nicotiana benthamiana*	Entire plants	Agrobacterium-mediated transformation	no data	[77]
IL-2	*Nicotiana tabacum*	Transplastomic plants	Bioballistic	0.005% total soluble protein	[84]
IL-3	*Nicotiana benthamiana*	Transient expression	Agroinfiltration	Maximum 0.144 mg/g	[123]
IL-4	*Nicotiana tabacum*	Suspension	Agrobacterium-mediated transformation	0.45 mg/L	[74]
IL-4	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	0.1% total soluble protein	[78]
IL-4	Tobacco	Entire plants	Agrobacterium-mediated transformation	0.086% total soluble protein	[127]
IL-4	*Solanum tuberosum*	Entire plants	Agrobacterium-mediated transformation	0.08% total soluble protein	[66]
IL-7	*Oryza sativa*	Entire plants	Agrobacterium-mediated transformation	5–10 μg/g	[83]
IL-10	Tobacco	Entire plants	Agrobacterium-mediated transformation	0.27% total soluble protein	[127]
IL-10	Tobacco	Entire plants	Agrobacterium-mediated transformation	Maximum 0.043 mg/g	[79]
IL-10	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	Maximum 0.037 mg/g	[94]
IL-10	*Oryza sativa*	Entire plants	Agrobacterium-mediated transformation	0.05 mg/g	[93]
IL-10	Tobacco, Arabidopsis	Mature plants	Agrobacterium-mediated transformation	0.7% total soluble protein	[128]
IL-10	*Nicotiana tabacum*	Suspension	Agrobacterium-mediated transformation	9.3 μg/g	[80]
IL-10	Tobacco	Suspension	Agrobacterium-mediated transformation	3.175% total soluble protein	[81]
IL-12	Tobacco	Suspension	Agrobacterium-mediated transformation	0.175 mg/L	[89]
IL-12	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	4×10^{-5} mg/g	[87]
IL-12	*Solanum lycopersicum*	Entire plants	Agrobacterium-mediated transformation	7.3×10^{-3} mg/g	[88]
IL-13	Tobacco	Entire plants	Agrobacterium-mediated transformation	0.15% total soluble protein	[90]
IL-18	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	Maximum 3.51×10^{-4} mg/g	[91]

Table 3. (Contd.)

Cytokine	Plant	Growing method	Transformation method	Content	Source
IL-18	Tobacco	Entire plants	Agrobacterium-mediated transformation	1.3×10^{-3} mg/g	[129]
IFN-α	*Solanum lycopersicum*	Suspension	Lipofectin-mediated transformation	Maximum 3.0×10^{-3} U/g	[130]
IFN-α2	*Aloe vera*	Entire plants	Bioballistic	Maximum 953 U/g	[131]
IFN-α2b	*Daucus carota*	Entire plants	Agrobacterium-mediated transformation	Maximum 26.8×10^{-3} U/g	[100]
IFN-α2b FN-α8	*Solanum tuberosum*	Entire plants	Agrobacterium-mediated transformation	560 U/g	[132]
IFN-α2b	*Nicotiana tabacum*	Transplastomic plants	Bioballistic	20% total soluble protein	[98]
Chicken IFN-α	*Lactuca sativa*	Transient expression	Agroinfiltration	0.393 mg/g	[133]
Salmo salar IFN-α1	*Solanum tuberosum*	Entire plants	Agrobacterium-mediated transformation	Maximum 5400 U/g	[101]
Salmo salar IFN-α1	*Oryza sativa*	Entire plants	Agrobacterium-mediated transformation	Maximum 820 U/g	[101]
IFN-β	*Lactuca sativa*	Transient expression	Agroinfiltration	3.1×10^{-4} U/mL	[134]
IFN-γ	*Oryza sativa*	Suspension	Agrobacterium-mediated transformation	6.99×10^{-4} mg/g cells	[102]
Bos taurus IFN-γ	*Nicotiana tabacum*	Entire plants	Agrobacterium-mediated transformation	1–1.5 μg/g	[99]
Fibroblast growth factor (FGF8b)	Tobacco	Entire plants	Agrobacterium-mediated transformation	4.1% total soluble protein	[106]
Human IGF-1	*Oryza sativa*	Mature plants	Bioballistic	6.8% total seed protein	[112]
Human IGF-1	Tobacco	Transplastomic plants	Bioballistic	Maximum 32% total soluble protein	[23]
Human IGF-1	*Nicotiana benthamiana*	Transient expression	Agroinfiltration	Maximum 0.25 mg/g	[123]
Human leukemia inhibitory factor (hLIF)	*Oryza sativa*	Entire plants	Bioballistic	No data	[135]
Tumor necrosis factor TNF-α	*Solanum tuberosum*	Entire plants	Agrobacterium-mediated transformation	0.015 mg/g	[105]
Cardiotrophin-1	Tobacco	Transplastomic plants	Bioballistic	Maximum 1.14 mg/g	[86]
Stem cell factor (SCF)	*Nicotiana benthamiana.*	Transient expression	Agroinfiltration	Maximum 0.2 mg/g	[123]

tor contained sequences, one of which encoded the N-terminal signal peptide and the other encoded the C-terminal peptide SEQDEL, which is responsible for the transport into the endoplasmic reticulum. However, this strategy did not lead to an increased accumulation of cytokines. The yield of the recombinant protein was about 15 mg of biologically active TNF-α per 1 g of plant tissue [105].

The biologically active human fibroblast growth factor 8 (FGF), which belongs to the platelet derived growth factor group (PDGF), was synthesized in tobacco plants. The cloning of cDNA *hFGF8b* was

performed under the control of the double 35S CaMV promoter. The FGF content was 2.7 and 4.1% of total soluble protein (90 and 150 mg/g fresh weight), respectively, in the system of transient expression after agroinfiltration in vacuum [106].

Insulin-like growth factor 1 (**IGF-1**), which belongs to the same family, was obtained in transplastomic plants [23]. An isolated recombinant protein had biological activity on human HU-3 cells.

Methods for improving the productivity of plant expression systems. The choice of promoter is an important step in the production of transgenic systems for recombinant protein synthesis. The most commonly constitutive promoters are used: 35S promoter derived from cauliflower mosaic virus and the ubiquitin promoter isolated from maize. The main advantage of constitutive promoters is the constant gene expression in all parts of the plant. However, constitutive promoters have a low expression level and often undergo silencing as a result of methylation during transgenic plant growth [107]. Methods based on the use of inducible promoters are activated only under special conditions, and tissue-specific promoters working in certain plant organs became more widely used with the development of biotechnology. The silencing phenomenon was not detected when inducible promoters were used. At the present time, there is no complete description of these promotors, which limits their use. The identified plant inducible promoters depend on steroids (estradiol) [108] and ethanol [109]. αAmy3/RAmy3D promoter, which is activated in the absence of sugar, and the promoter of the gene of the small heat shock protein 18.2 (sHSP18.2) are used in suspension cell cultures [62, 110, 111].

The use of promoters with specific expression patterns, particularly when a product accumulates in the seeds of transgenic plants, simplifies product isolation and can lead to an increased yield of the heterologous protein [68, 71, 93]. Thus, the combination of a strong endosperm-specific Gt13 promoter and codon optimization when expressed in rice cells led to the accumulation of human GM-CSF (14 μg/kg of seed). For protection against degradation by proteases, a signal peptide providing for the transport of the recombinant protein to the endoplasmic reticulum and protein bodies of endosperm cells was attached to the protein sequence [71]. Similar results were obtained by modifying a gene of insulin-like growth factor (IGF-1) and by attaching BipC protein, which is associated with the lumen of the endoplasmic reticulum of rice seeds to the C-terminal residue. The IGF yield in the seeds was more than 6% of the soluble protein [112]. Thus, the presence of intracellular transport signal can significantly increase the level of accumulation of the desired product as if it does not affect the functional properties of the protein.

The strategy of codon optimization in heterologous genes, which should correspond to the most common codon of the host, is also used in order to increase yield of the recombinant protein by the expression in the transgenic plant-based production systems. Codons become a limiting factor in protein translation when a foreign gene comprises the rare codons of the plant host. For example, after the optimization of transgene codons, an efficient accumulation of IGF-1 in transgenic tobacco chloroplasts was achieved. The native gene *IGF* (*IGF-n*), which contains 41% AT pairs, and the synthetic *IGF-s* gene, which has the optimal content of AT pairs for chloroplast (60%), were cloned into a vector with psbA promoter (promoter of the gene encoding a subunit of the reaction center of photosystem II), 5'-UTR (increases the translation efficiency during illumination), and 3'-UTR (increases the stability of the transcript). The accumulation of *IGF-n* product in the transgenic plants was 9.5% of soluble protein, and the accumulation of *IGF-s* product reached 11.3%. However, with continuous illumination, the IGF content increased up to 32% of the soluble proteins [23, 94].

One promising method for increased expression in plant systems involves a genetic modification of the host plant that decreases protease synthesis or restricts the silencing effect on the heterologous gene. In the first case, knockout of the genes encoding endogenous proteases occurs. Thus, the restriction of cysteine protease synthesis resulted in an increase in the production of human GM-CSF in rice cells by almost half (from 150.4 to 289.1 mg/L suspension) [64]. The knockout of endogenous α-amylase gene via RNA interference almost doubled its production with the use of αAmy3/RAmy3D promoter (from 150 to 280 mg/L) [63].

In the second case, knockout of the silencing mechanism responsible for the reduction in the expression level of the recombinant protein is provided. In order to achieve this, *Nicotiana benthamiana* plants were infected with tomato bushy stunt virus (**TBSV**), which "diverted" the silencing system. The yield of murine immunoglobulin class G increased 14 times (from 10.8 to 147.7 mg/g fresh weight of leaves) [113, 114].

The third method of increasing the productivity of the plant expression systems is the manipulation of glycosylation mechanisms of heterologous proteins. An initial failure in the production of functionally active heterologous proteins in transgenic plants, in particular erythropoietin [57], confirmed the assumption that the difference in the composition of the glycosidic groups of native mammalian glycoproteins and proteins expressed in plants determine the stability of the recombinant product and its biological function [115]. Attachment of the carbohydrates to the polypeptide chain occurs in the endoplasmic reticulum and Golgi apparatus. Although many steps of protein N-glycosylation are common to all eukaryotes, the composition of complex N-glycans in plants and

mammals may be different [116]. Thus, the C-terminal residue of the polysaccharide chains of human glycoproteins in most cases is a sialic acid residue, but plants lack a sialylation mechanism. Plants also synthesize glycans, which contain residues of sugars such as xylose, rhamnose, and arabinose [8] that are not typical for human glycoproteins. Polysaccharide chains of plant glycans contain β-1,2-xylose and α-1,3-fucose residues in lateral branches, whereas xylose residues are absent in mammalian glycans and fucose is present in the form of α-1,6-fucose. Finally, mucin-type O-glycosylation, which is characteristic for many secreted mammalian glycoproteins, is not operating in plants [117]. Thus, differences in the glycosylation mechanism of plants and humans can lead to a loss of the biological function of recombinant products, reduce their half-life in the blood, and induce an immune response in the recipient organism [8, 111].

Transgenic plants with a "humanized" glycosylation system were produced to solve this problem. There are several strategies for changes to the post-translational modification of the recombinant protein expressed in plants that contribute to the production of a structure close to the native form. One promising approach is to modify the recombinant protein by the attachment of the KDEL amino acid sequence, which is responsible for protein localization to the endoplasmic reticulum, to the C-terminal residue. This prevents targeting of the protein in the Golgi apparatus, where specific plant fucosyl- and xylosyltransferases are localized, and results in the production of a structure of the glycoside residue of the recombinant protein similar to the structure to the human protein. The disadvantage of this approach is that it is impossible to direct the protein from the endoplasmic reticulum to the other cellular compartments or for secretion into the culture medium. In this case, the cell-destruction stage for product recovery becomes mandatory [111]. Recombinant IL-10 was produced in rice plants by this method [93].

Another approach involves the use of the mutant moss *Physcomitrella patens* (Hedw.) Bruch & Schimp. plants as a producer. The distinguishing feature of *P. patens* is its high frequency of homologous recombination, which allows highly accurate knockout of α1,3-fucosyltransferase and β-1,2-xylosyltransferase genes [118]. The recombinant erythropoietin was produced using Δ*fuc-t* Δ*-xyl-t P. patens* mutant line [58]. In another study [119] for the production of recombinant GM-CSF in the rice suspension cell culture 1,3-fucosyltransferase and β-1,2-xylosyltransferase genes were knocked out by RNA interference, and the cells did not lose viability. However, for fully valid plants, defects of genes encoding specific xylosyltransferases may lead to reduced viability of cells and the appearance of phenotype abnormalities [111].

Diverse approach involves the production of glycosylation systems of mammalian proteins in plant cells [120]. Thus, for the production of recombinant EPO transgenic tobacco plants lacking plant glycosyltransferase and expressing genes encoding mammalian enzymes, β-1,4-mannosyl-β, 4-N-acetylglucosamynyltransferase (GnTIII), α-1,3-mannosyl-β-1,4-N-acetylglucosamynyltransferase (GnTIV) and α-1,6-mannosyl-β-1,6-N-acetylglucosamynyltransferase (GnTV) were used. This led to the ligation of N-glycans typical to human proteins to the recombinant glycoprotein [121]. The presence of sialic acid residues in glycan chains affected the stability and bioactivity of recombinant EPO. Various glycoforms of biologically active bi- and multisialylated EPO were obtained from mutant Δ*-fuc-t* Δ*-xyl-t* tobacco plants carrying genes for the branching of polysaccharide chains, β-1,4-galactosylation, and the synthesis and transport of sialic acid [120].

CONCLUSIONS

Thus, transgenic plants are promising media for the synthesis of recombinant proteins for medical purposes. For a long time, the low level of heterologous protein synthesis prevented the commercialization of plant systems for the production of biopharmaceutical products, but the achievements of the last decade have substantially improved their productivity. The use of transient or transplastomic expression and effective promoters, as well as the optimization of codons and cultivation of cells in suspension culture, significantly increases the yield of recombinant protein. Directing the recombinant protein to the endoplasmic reticulum and the vacuole as a result of the attachment of the signal of intracellular transport to the protein also makes it possible to avoid proteolysis and glycosylation via pathways specific for plants that are different from the human pathway, contributing to the improvement of synthesis efficiency. Decreased intracellular enzyme activity via knockout of the corresponding genes or coexpression of proteinase inhibitors was also used to increase the target protein yield. Modifications of transgenic bioreactor plants that change the system of post-translational modification of the protein allows the production of complex recombinant animal and human glycoproteins most similar to native types in terms of biological activity and allergic reactions. A major success in the production of plants with humanized expression system has been achieved for such model plants as Arabidopsis, tobacco, and *Physcomitrella patens*. The first steps for the modification of glycosylation systems of cereals and legumes, which are the most efficient producers, were taken.

The development of new transformation methods allows the expectation of significant progress in the production of transgenic organisms in the nearest future. Such DNA-binding protein domains as "zinc finger" or transcription activator-like (TAL), which is attached to restriction enzyme (ZFNs-zinc finger

nucleases), and transcription activator-like effector nucleases (TALEN-nucleases) will help in the editing of specific regions of the genome with the high accuracy. In combination with genome-wide sequencing technologies, ZFN and TALEN are promising molecular tools for genetic engineering that open the possibility of gene target changes in different types of cells and organisms [122].

At the present time, our understanding the nature of many diseases of the immune system has contributed to a significant increase in the global demand for cytokines, which are used for the treatment of a wide range of socially significant oncology, infectious, immunodeficiency diseases, and other conditions [38]. The introduction of the first plant recombinant cytokine, Locteron®, into the market [104] clears the path for further development of this field of pharmacology. To date, the synthesis of representatives of almost all families of cytokines preserving their biological activity was performed with transgenic plants. New technologies of genome editing and strategies for increasing the level of expression of recombinant proteins in plants offer opportunities for low-cost, large-scale production of cytokines with improved product quality and post-translational modifications of the type characteristic for humans and the higher animals. These technologies can be used to overcome some of the problems of global public health and veterinary medicine.

ACKNOWLEGMENTS

The work was supported by the development program of St. Petersburg State University (project 1.38.229.2014).

REFERENCES

1. Itakura, K., Hirose, T., Crea, R., Riggs, A., Heyneker, H., Bolivar, F., and Boyer, H., *Science*, 1977, vol. 198, no. 4308, pp. 1056–1063.
2. Assenberg, R., Wan, P., Geisse, S., and Mayr, L., *Curr. Opin. Struct. Biol.*, 2013, vol. 23, no. 3, pp. 393–402.
3. Lambertz, C., Garvey, M., Klinger, J., Heesel, D., Klose, H., Fischer, R., and Commandeur, U., *Biotechnol. Biofuels*, 2014, vol. 7, no. 1, p. 135.
4. Padkina, M.V., Parfenova, L.V., Gradoboeva, A.E., and Sambuk, E.V., *Appl. Biochem. Microbiol.*, 2010, vol. 46, no. 4, pp. 409–414.
5. Fahad, S., Khan, F., Pandupuspitasari, N., Ahmed, M., Liao, Y., Waheed, M., Sameeullah, M., Darkhshan, HussainS., Saud, S., Hassan, S., Jan, A., Jan, M., Wu, C., Chun, M., and Huang, J., *Biotechnol. Lett.*, 2015, vol. 37, no. 2, pp. 265–279.
6. Bosze, Z., Baranyi, M., and Whitelaw, C., *Adv. Exp. Med. Biol.*, 2008, vol. 606, pp. 357–393.
7. Li, J., Gu, L., Aach, J., and Church, G., *PLoS ONE*, 2014, vol. 9, no. 9, p. e106232. doi: 10.1371/journal.pone.0106232
8. Brooks, S.A., *Mol. Biotechnol.*, 2004, vol. 28, no. 3, pp. 241–255.
9. Rosano, G. and Ceccarelli, E., *Front. Microbiol.*, 2014, vol. 5, p. 172. doi: 10.3389/fmicb.2014.00172.
10. Bevan, M., Flavell, R., and Chilton, M.D., *Biotechnology*, 1983, vol. 24, pp. 367–370.
11. Ronald, P., *Genetics*, 2011, vol. 188, no. 1, pp. 11–20.
12. Rukavtseva, E.B., Bur'yanov, Ya.I., Shul'ga, N.Ya., and Bykov, V.A., *Vopr. Biol. Med. Farm.Khim.*, 2006, no. 2, pp. 3–12.
13. Deineko, E.V., *Vestn. Tomsk. Gos. Univ., Ser. Biol.*, 2012, no. 2, pp. 41–51.
14. Barta, A., Sommergruber, K., Thompson, D., Hartmuth, K., Matzke, M., and Matzke, A.J.M., *Plant. Mol. Biol.*, 1986, vol. 6, no. 5, pp. 347–357.
15. Hiatt, A., Cafferkey, R., and Bowdish, K., *Nature*, 1989, vol. 342, no. 6245, pp. 76–78.
16. Hood, E., Witcher, D., Maddock, S., Meyer, T., Baszczynski, C., Bailey, M., Flynn, P., Register, J., Marshall, L., Bon, D., Kulisek, E., Kusnadi, A., Evangelista, R., Nikolov, Z., Wooge, C., Mehig, R., Hernan, R., Kappel, W., Ritland, D., Li, C., and Howard, J., *Mol. Breed.*, 1997, vol. 3, no. 4, pp. 291–306.
17. Obembe, O., Popoola, J., Leelavathi, S., and Reddy, S., *Biotechnol. Adv.*, 2011, vol. 29, no. 2, pp. 210–222.
18. Gomord, V., Fitchette, A., Menu-Bouaouiche, L., Saint-Jore-Dupas, C., Plasson, C., Michaud, D., and Faye, L., *Plant Biotechnol. J.*, 2010, vol. 8, no. 5, pp. 564–587.
19. Xu, J., Ge, X., and Dolan, M., *Biotechnol. Adv.*, 2011, vol. 29, no. 3, pp. 278–299.
20. Lienard, D., Sourrouille, C., Gomord, V., and Faye, L., *Biotechnol. Annu. Rev.*, 2007, vol. 13, pp. 115–147.
21. Kim, H.J., Lee, D.H., Kim, D.K., Han, G.-B., and Kim, H.-J., *Biol. Pharm. Bull.*, 2008, vol. 31, no. 2, pp. 290–294.
22. Desai, P., Shrivastava, N., and Padh, H., *Biotechnol. Adv.*, 2010, vol. 28, no. 4, pp. 427–435.
23. Daniell, H., Ruiz, G., Denes, B., Sandberg, L., and Langridge, W., *BMC. Biotechnol.*, 2009, vol. 9, p. 33. doi: 10.1186/1472-6750-9-33.
24. Lutova, L.A., *Biotekhnologiya vysshikh rastenii* (Biotechnology of Higher Plants), St. Petersburg: Izd. St.-Peterburg. Univ., 2003.
25. Broothaerts, W., Mitchell, H.J., Weir, B., Kaines, S., Smith, L.M.A., Yang, W., Mayer, J.E., Roa-Rodrıguez, C., and Jefferson, R.A., *Nature*, 2005, vol. 433, no. 7026, pp. 629–633.
26. Gao, C. and Nielsen, K.K., *Methods Mol. Biol.*, 2013, vol. 940, pp. 3–16.
27. Sujatha, M. and Visarada, K.B., *Methods Mol. Biol.*, 2013, vol. 940, pp. 27–44.
28. Oey, M., Lohse, M., Kreikemeyer, B., and Bock, R., *Plant J.*, 2009, vol. 57, no. 3, pp. 436–445.
29. Larosa, V. and Remacle, C., *Int. J. Dev. Biol.*, 2013, vol. 57, nos. 6–8, pp. 659–665.
30. Kittur, F.S., Bah, M., Archer-Hartmann, S., Hung, C.-Y., Azadi, P., Ishihara, M., Sane, D.C., and Xie, J., *PLoS ONE*, 2013, vol. 8, no. 10, p. e76468. doi: 10.1371/journal.pone.0076468.
31. Kanagarajan, S., Tolf, C., Lundgren, A., Waldenstrom, J., and Brodelius, P.E., *PLoS ONE*, 2012,

vol. 7, no. 3, p. e33010. doi: 10.1371/journal.pone.0033010.

32. Hefferon, K.L., *Open Access Scientific Reports*, 2012, vol. 1, no. 2, p. 174. doi:10.4172/scientificreports.174.
33. Gleba, Y.Y., Tuse, D., and Giritch, A., *Curr. Top. Microbiol. Immunol.*, 2014, vol. 375, pp. 155–192.
34. Gleba, Y., Klimyuk, V., and Marillonnet, S., *Vaccine*, 2005, vol. 23, nos. 17–18, pp. 2042–2048.
35. Tremblay, R., Wang, D., Jevnikar, A.M., and Ma, S., *Biotechnol. Adv.*, 2010, vol. 28, no. 2, pp. 214–221.
36. Stiles, A. and Liu, C., *Adv. Biochem. Engineer. Biotechnol.*, 2013, vol. 134, pp. 91–114.
37. Huang, T.-K. and McDonald, K.A., *Biotechnol. Adv.*, 2012, vol. 30, no. 2, pp. 398–409.
38. Paul, M.J., Teh, A.Y.H., Twyman, R.M., and Ma, J.K.-C., *Curr. Pharmaceut. Des.*, 2013, vol. 19, no. 31, pp. 5478–5485.
39. Cunha, N.B., Vianna, G.R., Almeida, LimaT., and Rech, E., *Biotechnol. J.*, 2014, vol. 9, no. 1, pp. 39–50.
40. Rybicki, E.P., *Virology J.*, 2014, vol. 11, Art. 205. doi: 10.1186/s12985-014-0205-0.
41. Burnouf, T., *Vox Sang*, 2011, vol. 100, no. 1, pp. 68–83.
42. Simbirtsev, A.S., *Med. Akad. Zh.*, 2013, vol. 13, no. 1, pp. 7–22.
43. Khaitov, R.M., Ignat'eva, G.A., and Sidorovich, I.G., *Immunologiya* (Immunology), Moscow: Meditsina, 2000.
44. Sirko, A., Vanek, T., Gora-Sochacka, A., and Redkiewicz, P., *Int. J. Mol. Sci.*, 2011, vol. 12, no. 6, pp. 3536–3552.
45. Xie, Y.F., Chen, H., and Huang, B.R., *J. Biotechnol.*, 2007, vol. 129, no. 3, pp. 472–480.
46. Avdeeva, Zh.I., Alpatova, N.A., Akol'zina, S.E., and Medunitsyn, N.V., *Tikhookean. Med. Zh.*, 2009, no. 3, pp. 19–21.
47. Simbirtsev, A.S., Petrov, A.V., Pigareva, N.V., and Nikolaev, A.T., *Biopreparaty*, 2011, no. 1, pp. 16–20.
48. Sayers, S., Ulysse, G., Xiang, Z., and He, Y., *J. Biomed. Biotechnol.*, 2012, vol. 2012, p. 831486. doi: 10.1155/2012/831486.
49. Baldo, B.A., *Drug Safety*, 2014, vol. 37, no. 11, pp. 921–943.
50. Gradoboeva, A.E. and Padkina, M.V., *Vestnik SPbGU, Ser. 3*, 2008, no. 2, pp. 56–61.
51. Tsygankov, M.A., Zobnina, A.E., and Padkina, M.V., *Appl. Biochem. Microbiol.*, 2014, vol. 50, no. 4, pp. 387–393.
52. Karabel'skii, A.V., Zinov'eva, Yu.G., Smirnov, M.N., and Padkina, M.V., *Vestnik SPbGU, Ser. 3*, 2009, no. 2, pp. 53–63.
53. Karabel'skii, A.V. and Padkina, M.V., *Appl. Biochem. Microbiol.*, 2012, vol. 48, no. 4, pp. 416–420.
54. Flynn, J.L., Chan, J., Triebold, K.J., Dalton, D.K., Stewart, T.A., and Bloom, B.R., *J. Exp. Med.*, 1993, vol. 178, no. 6, pp. 2249–2253.
55. Vesosky, B., Flaherty, D.K., and Turner, J., *Infect. Immun.*, 2006, vol. 74, no. 6, pp. 3314–3324.
56. Ershov, F.I., *Sistema interferona v norme i pri patologii* (Interferon System in Health and Disease), Moscow: Meditsina, 1996.
57. Matsumoto, S., Ikura, K., Ueda, M., and Sasaki, R., *Plant. Mol. Biol.*, 1995, vol. 27, no. 6, pp. 1163–1172.
58. Weise, A., Altmann, F., Rodriguez-Franco, M., Sjoberg, E.R., Baumer, W., Launhardt, H., Kietzmann, M., and Gorr, G., *Plant Biotechnol. J.*, 2007, vol. 5, no. 3, pp. 389–401.
59. Cheon, B.Y., Kim, H.J., Oh, K.H., Bahn, S.C., Ahn, J.H., Choi, J.W., Ok, S.H., Bae, J.M., and Shin, J.S., *Transgenic Res.*, 2004, vol. 13, no. 6, pp. 541–549.
60. James, E.A., Wang, C., Wang, Z., Reeves, R., Shin, J.H., Magnuson, N.S., and Lee, J.M., *Protein Expr. Purif.*, 2000, vol. 19, no. 1, pp. 131–138.
61. Lee, J.-H., Kim, N.-S., Kwon, T.-H., Jang, Y.-S., and Yang, M.-S., *J. Biotechnol.*, 2002, vol. 96, no. 3, pp. 205–211.
62. Shin, Y.-J., Hong, S.-Y., Kwon, T.-H., Jang, Y.-S., and Yang, M.-S., *Biotechnol. Bioeng.*, 2003, vol. 82, no. 7, pp. 778–783.
63. Kim, N.-S., Kim, T.-G., Jang, Y.-S., Shin, Y.J., Kwon, T.-H., and Yang, M.-S., *Plant. Mol. Biol.*, 2008, vol. 68, nos 4-5, pp. 369–377.
64. Kim, T.-G., Lee, H.-J., Jang, Y.-S., Shin, Y.-J., Kwon, T.-H., and Yang, M.-S., *Protein Expr. Purif.*, 2008, vol. 61, no. 2, pp. 117–121.
65. Liu, Y.-K., Huang, L.-F., Ho, S.-L., Liao, C.-Y., Liu, H.-Y., Lai, Y.-H., Yu, S.-M., and Lu, C.-A., *Biotechnol. Bioeng.*, 2012, vol. 109, no. 5, pp. 1239–1247.
66. Gora-Sochacka, A., Redkiewicz, P., Napiorkowska, B., Gaganidze, D., Brodzik, R., and Sirko, A., *J. Interferon Cytokine Res.*, 2010, vol. 30, no. 3, pp. 135–142.
67. Wang, M.-L., Goldstein, C., Su, W., Moore, P.H., and Albert, H.H., *Transgenic Res.*, 2005, vol. 14, no. 2, pp. 167–178.
68. Sardana, R., Dudani, A.K., Tackaberry, E., Alli, Z., Porter, S., Rowlandson, K., Ganz, P., and Altosaar, I., *Transgenic Res.*, 2007, vol. 16, no. 6, pp. 713–721.
69. Sardana, R.K., Alli, Z., Dudani, A., Tackaberry, E., Panahi, M., Narayanan, M., Ganz, P., and Altosaar, I., *Transgenic Res.*, 2002, vol. 11, no. 5, pp. 521–531.
70. Zhou, F., Wang, M.-L., Albert, H.H., Moore, P.H., and Zhu, Y.J., *Appl. Microbiol. Biotechnol.*, 2006, vol. 72, no. 4, pp. 756–762.
71. Ning, T., Xie, T., Qiu, Q., Yang, W., Zhou, S., Zhou, L., Zheng, C., Zhu, Y., and Yang, D., *Biotechnol. Lett.*, 2008, vol. 30, no. 9, pp. 1679–1686.
72. Won, J.H., Ji, J.E., Ahn, K.H., Kim, S.K., Choi, J.M., Ha, H.C., Kim, H.M., Yun, C.K., Han, K., and Kim, D.K., *Biol. Pharmaceut. Bull.*, 2013, vol. 36, no. 3, pp. 425–431.
73. Nikitina, T.N. and Avdeeva, Zh.I., *Biopreparaty*, 2008, no. 1, pp. 16–20.
74. Magnuson, N.S., Linzmaier, P.M., Reeves, R., An, G., HayGlass, K., and Lee, J.M., *Protein Expr. Purif.*, 1998, vol. 13, no. 1, pp. 45–52.
75. Park, Y. and Cheong, H., *Protein Expr. Purif.*, 2002, vol. 25, no. 1, pp. 160–165.
76. Redkiewicz, P., Wiesyk, A., Gora-Sochacka, A., and Sirko, A., *Plant Biotechnol. J.*, 2012, vol. 10, no. 7, pp. 806–814.

77. Matakas, J.D. and Balan, V., Carson IV, W.F., Gao, D., Brandizzi, F., Kunkel, S., and Sticklen, M., *Int. J. Life Sci. Biotechnol. Pharma Res.*, 2013, vol. 2, no. 2, pp. 192–203.

78. Ma, S., Huang, Y., Davis, A., Yin, Z., Mi, Q., Menassa, R., Brandle, J.E., and Jevnikar, A.M., *Plant Biotechnol. J.*, 2005, vol. 3, no. 3, pp. 309–318.

79. Menassa, R., Kennette, W., Nguyen, V., Rymerson, R., Jevnikar, A., and Brandle, J., *J. Biotechnol.*, 2004, vol. 108, no. 2, pp. 179–183.

80. Bortesi, L., Rademacher, T., Schiermeyer, A., Schuster, F., Pezzotti, M., and Schillberg, S., *BMC Biotechnol.*, 2012, vol. 12, Art. 40. doi: 10.1186/1472-6750-12-40.

81. Kaldis, A., Ahmad, A., Reid, A., McGarvey, B., Brandle, J., Ma, S., Jevnikar, A., Kohalmi, S.E., and Menassa, R., *Plant Biotechnol. J.*, 2013, vol. 11, no. 5, pp. 535–545.

82. Chen, L., Dempsey, B.R., Gyenis, L., Menassa, R., Brandle, J.E., and Dhaubhadel, S., *Plant Biotechnol. J.*, 2013, vol. 11, no. 5, pp. 546–554.

83. Kudo, F., Ohta, M., Yang, L., Wakasa, Y., Takahashi, S., and Takaiwa, F., *Plant. Mol. Biol.*, 2013, vol. 81, nos. 4–5, pp. 461–475.

84. Zhang, X.-H., Keating, P., Wang, X.-W., Huang, Y.-H., Martin, J., Hartmann, J.X., and Liu, A., *Mol. Biotechnol.*, 2014, vol. 56, no. 4, pp. 369–376.

85. De Marchis, F., Pompa, A., and Bellucci, M., *Plant Physiol.*, 2012, vol. 160, no. 2, pp. 571–581.

86. Farran, I., Rio-Manterola, F., Iniguez, M., Garate, S., Prieto, J., and Mingo-Castel, A.M., *Plant Biotechnol. J.*, 2008, vol. 6, no. 5, pp. 516–527.

87. Gutierrez-Ortega, A., Avila-Moreno, F., Saucedo-Arias, L.J., Sanchez-Torres, C., and Gomez-Lim, M.A., *Biotechnol. Bioeng.*, 2004, vol. 85, no. 7, pp. 734–740.

88. Gutierrez-Ortega, A., Sandoval-Montes, C., de Olivera-Flores, T.J., Santos-Argumedo, L., and Gomez-Lim, M.A., *Transgenic Res.*, 2005, vol. 14, no. 6, pp. 877–885.

89. Kwon, T.H., Seo, J.E., Kim, J., Lee, J.H., Jang, Y.S., and Yang, M.S., *Biotechnol. Bioeng.*, 2003, vol. 81, no. 7, pp. 870–875.

90. Wang, D.J., Brandsma, M., Yin, Z., Wang, A., Jevnikar, A.M., and Ma, S., *Plant Biotechnol. J.*, 2008, vol. 6, no. 5, pp. 504–515.

91. Zhang, B., Yang, Y., Lin, Y., Rao, Q., Zheng, G., and Wu, K., *Biotechnol. Lett.*, 2003, vol. 25, no. 19, pp. 1629–1635.

92. Medrano, J., Reidy, M., Liu, J., Ayala, M., Dolan, M., and Cramer, C., *Methods Mol. Biol.*, 2009, vol. 483, pp. 41–67.

93. Fujiwara, Y., Aiki, Y., Yang, L., Takaiwa, F., Kosaka, A., Tsuji, N.M., Shiraki, K., and Sekikawa, K., *Protein Expr. Purif.*, 2010, vol. 72, no. 1, pp. 125–130.

94. Bortesi, L., Rossato, M., Schuster, F., Raven, N., Stadlmann, J., Avesani, L., Falorni, A., Bazzoni, F., Bock, R., Schillberg, S., and Pezzotti, M., *BMC Biotechnol.*, 2009, vol. 9, Art. 22. doi:10.1186/1472-6750-9-22.

95. Schroder, K., Hertzog, P.J., Ravasi, T., and Hume, D.A., *J. Leukocyte Biol.*, 2004, vol. 75, no. 2, pp. 163–189.

96. Fensterl, V. and Sen, G.C., *BioFactors*, 2009, vol. 35, no. 1, pp. 14–20.

97. Edelbaum, O., Stein, D., Holland, N., Gafni, Y., Livneh, O., Novick, D., Rubinstein, M., and Sela, I., *J. Interferon Res.*, 1992, vol. 12, no. 6, pp. 449–453.

98. Arlen, P.A., Falconer, R., Cherukumilli, S., Cole, A., Cole, A.M., Oishi, K.K., and Daniell, H., *Plant Biotechnol. J.*, 2007, vol. 5, no. 4, pp. 511–525.

99. Saveleva, N.V., Kurdyukov, I.D., Dudnik, E.E., Yemelyanov, V.V., Padkina, M.V., and Lutova, L.A., *Vestnik SPbGU, Ser. 3*, 2009, no. 4, pp. 65–80.

100. Luchakivskaya, Yu., Kishchenko, O., Gerasymenko, I., Olevinskaya, Z., Simonenko, Yu., Spivak, M., and Kuchuk, M., *Plant Cell Rep.*, vol. 30, no. 3, pp. 407–415.

101. Fukuzawa, N., Tabayashi, N., Okinaka, Y., Furusawa, R., Furuta, K., Kagaya, U., and Matsumura, T., *J. Biosci. Bioeng.*, 2010, vol. 110, no. 2, pp. 201–207.

102. Chen, T.-L., Lin, Y.-L., Lee, Y.-L., Yang, N.-S., and Chan, M.-T., *Transgenic Res.*, 2004, vol. 13, no. 5, pp. 499–510.

103. RF Patent no. 2468082, 2012.

104. De Leede, L.G., Humphries, J.E., Bechet, A.C., Van Hoogdalem, E.J., Verrijk, R., and Spencer, D.G., *J. Interferon Cytokine Res.*, 2008, vol. 28, no. 2, pp. 113–122.

105. Ohya, K., Itchoda, N., Ohashi, K., Onuma, M., Sugimoto, C., and Matsumura, T., *J. Interferon Cytokine Res.*, 2002, vol. 22, no. 3, pp. 371–378.

106. Potula, H.H.S.K., Kathuria, S.R., Ghosh, A.K., Maiti, T.K., and Dey, S., *Transgenic Res.*, 2008, vol. 17, no. 1, pp. 19–32.

107. Lee, G.-H., Sohn, S.-H., Park, E.-Y., and Park, Y.-D., *Funct. Plant Biol.*, 2012, vol. 39, no. 9, pp. 764–773.

108. Okuzaki, A., Konagaya, K.-I., Nanasato, Y., Tsuda, M., and Tabei, Y., *Plant Cell Rep.*, 2011, vol. 30, no. 4, pp. 529–538.

109. Garoosi, G.A., Salter, M.G., Caddick, M.X., and Tomsett, A.B., *J. Exp. Bot.*, 2005, vol. 56, no. 416, pp. 1635–1642.

110. Lee, K.-T., Chen, S.-C., Chiang, B.-L., and Yamakawa, T., *Appl. Microbiol. Biotechnol.*, 2007, vol. 73, no. 5, pp. 1047–1053.

111. Kuo, Y.-C., Tan, C.-C., Ku, J.-T., Hsu, W.-C., Su, S.-C., Lu, C.-A., and Huang, L.-F., *Int. J. Mol. Sci.*, 2013, vol. 14, no. 5, pp. 8719–8739.

112. Xie, T., Qiu, Q., Zhang, W., Ning, T., Yang, W., Zheng, C., Wang, C., Zhu, Y., and Yang, D., *Peptides*, 2008, vol. 29, no. 11, pp. 1862–1870.

113. Voinnet, O., Rivas, S., Mestre, P., and Baulcombe, D., *Plant J.*, 2003, vol. 33, no. 5, pp. 949–956.

114. Boivin, E.B., Lepage, E., Matton, D.P., De Crescenzo, G., and Jolicoeur, M., *Biotechnol. Prog.*, 2010, vol. 26, no. 6, pp. 1534–1543.

115. Paulus, K.E., Mahler, V., Pabst, M., Kogel, K.-H., Altmann, F., and Sonnewald, U., *Front. Plant Sci.*, 2011, vol. 2, Art. 42. doi: 10.3389/fpls.2011.00042.

116. Castilho, A., Neumann, L., Gattinger, P., Strasser, R., Vorauer-Uhl, K., Sterovsky, T., Altmann, F., and Steinkellner, H., *PLoS ONE*, 2013, vol. 8, no. 1, p. e54836. doi:10.1371/journal.pone.0054836.

117. Castilho, A., Neumann, L., Daskalova, S., Mason, H.S., Steinkellner, H., Altmann, F., and Strasser, R., *J. Biol. Chem.*, 2012, vol. 287, no. 43, pp. 36518–36526.

118. Koprivova, A., Stemmer, C., Altmann, F., Hoffmann, A., Kopriva, S., Gorr, G., Reski, R., and Decker, E.L., *Plant Biotechnol. J.*, 2004, vol. 2, no. 6, pp. 517–523.

119. Shin, Y.J., Chong, Y.-J., Yang, M.-S., and Kwon, T.-H., *Plant Biotechnol. J.*, 2011, vol. 9, no. 9, pp. 1109–1119.

120. Jez, J., Castilho, A., Grass, J., Vorauer-Uhl, K., Sterovsky, T., Altmann, F., and Steinkellner, H., *Biotechnol. J.*, 2013, vol. 8, no. 3, pp. 371–382.

121. Castilho, A., Gattinger, P., Grass, J., Jez, J., Pabst, M., Altmann, F., Gorfer, M., Strasser, R., and Steinkellner, H., *Glycobiology*, 2011, vol. 21, no. 6, pp. 813–823.

122. Joung, J.K. and Sander, J.D., *Nat. Rev. Mol. Cell Biol.*, 2013, vol. 14, no. 1, pp. 49–55.

123. Musiychuk, K., Sivalenka, R., Jaje, J., Bi, H., Flores, R., Shaw, B., Jones, R.M., Golovina, T., Schnipper, J., Khandker, L., Sun, R., Li, C., Kang, L., Voskinarian-Berse, V., Zhang, X., Streatfield, S., Hambor, J., Abbot, S., and Yusibov, V., *Stem Cells Dev.*, 2013, vol. 22, no. 16, pp. 2326–2340.

124. Tabar, M.S., Habashi, A.A., and Memari, H.R., *Iran. Biomed. J.*, 2013, vol. 17, no. 3, pp. 158–164.

125. Kwon, T.-H., Kim, Y.-S., Lee, J.-H., and Yang, M.-S., *Biotechnol. Lett.*, 2003, vol. 25, no. 18, pp. 1571–1574.

126. Zhao, Y., Qian, Q., Wang, H., and Huang, D., *J. Genet. Genomics*, 2007, vol. 34, no. 9, pp. 824–835.

127. Patel, J., Zhu, H., Menassa, R., Gyenis, L., Richman, A., and Brandle, J., *Transgenic Res.*, 2007, vol. 16, no. 2, pp. 239–249.

128. Morandini, F., Avesani, L., Bortesi, L., Van Droogenbroeck, B., De Wilde, K., Arcalis, E., Bazzoni, F., Santi, L., Brozzetti, A., Falorni, A., Stoger, E., Depicker, A., and Pezzotti, M., *Plant Biotechnol. J.*, 2011, vol. 9, no. 8, pp. 911–921.

129. RF Patent no. 2302460, 2007.

130. Sawahel, W.A., *Cell. Mol. Biol. Lett.*, 2002, vol. 7, no. 1, pp. 19–29.

131. Lowther, W., Lorick, K., Lawrence, S.D., and Yeow, W.-S., *Transgenic Res.*, 2012, vol. 21, no. 6, pp. 1349–1357.

132. Ohya, K., Matsumura, T., Ohashi, K., Onuma, M., and Sugimoto, C., *J. Interferon Cytokine Res.*, 2001, vol. 21, no. 8, pp. 595–602.

133. Song, L., Zhao, D.-G., Wu, Y.-J., and Li, Y., *J. Zhejiang. Univ. Sci. B*, 2008, vol. 9, no. 5, pp. 351–355.

134. Li, J., Chen, M., Liu, X.-W., Zhang, H.-C., Shen, F.F., and Wang, G.P., *Sci. Hortic.*, 2007, vol. 112, no. 3, pp. 258–265.

135. Youngblood, B.A., Alfano, R., Pettit, S.C., Zhang, D., Dallmann, H.G., Huang, N., and Macdonald, C.C., *J. Biotechnol.*, 2014, vol. 172, pp. 67–72.

Translated by V. Mittova