

Genetically modified potato plants in nutrition and prevention of diseases in humans and animals: a review

R. PRIBYLOVA, I. PAVLIK, M. BARTOS

Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Genetically modified organisms (GMO) become a real constituent of our lives and nowadays, they are commonly introduced into the food chain of people and animals in some states. Among higher organisms, plants are used above all for genetic modifications; potatoes are a suitable model plants for this purpose. Nowadays, a number of various genetic modifications of potato plants are available, particularly those with increased resistance to biological agents and factors of the external environment or with improved nutritional value. Plants that produce proteins of the immune system of man or animals or substances that may be used as vaccines in human or veterinary medicine are highly important. Modified potato plants that produce biomaterials for potential applications in the industry are a significant category.

Keywords: genetically modified organisms (GMO); genetic modification; transformation; transgene; plants; *Solanum tuberosum*; nutrition

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1. Introduction

Genetically modified organism (GMO) is a viable organism whose genetic information has been changed by genetic technology. Among higher organisms, plants are particularly used for genetic modifications. In the sphere of genetic modifications, potato plants represent a suitable model plant for several reasons. Their undisputable advantage, in contrast to other model plants, is the presence of tubers which serve as a resource of carbon and nitrogen. Moreover, potato plants propagate vegetatively, and thus a particular feature may be conserved for a long time. On the other hand, its drawback includes a tetraploid genome, low numbers of produced mutants and a relatively high variability. Cultivar Desirée is usually used in Europe for investigations focused on expression of foreign genes in plants (Davies, 1996).

A relatively large number of various genetically modified potato plants exist at present. The aim of this review is not to exhaustively describe all of them, but to mention the most valid and noteworthy ones from an aspect of agricultural production, human and veterinary medicine and alternative production of biopolymers.

2. Potato plants with increased resistance

A number of improved cultivars of agricultural plants are less naturally resistant in comparison with their progenitors. This problem appeared above all due to the introduction of plant monocultures, which resulted in vitalization of pests. However, extensive application of chemical pesticides led to a novel type resistance in the pest population. From this aspect, a production of resistant crops

contributing to reduction of consequences caused by pests and decreased use of chemical insecticides might be beneficial (Slater et al., 2003).

2.1. Insects

2.1.1. Potato carrying a gene *Cry3A* from *Bacillus thuringiensis*

One of the most consequential potato plant pests is the potato beetle (*Leptinotarsa decemlineata*), which often becomes resistant to chemical insecticides. Modified potatoes carrying gene *Cry3A* originating from bacteria *Bacillus thuringiensis* were produced to control this beetle. This gene product is a toxic protein formed in leaves of these plants; after ingestion by a potato beetle, it passes on to its intestines and thus causes the death of the pest. It is a great advantage that the protein affects all developmental stages of potato beetles in the same way; however it does not affect their natural enemies (Perlak et al., 1993).

2.1.2. Potato carrying a gene for lectins from snowdrop

However above mentioned modification is highly selective it does not sustain protection of a plant against other pest categories. Accordingly, potatoes carrying genes for the production of other insecticide proteins have been developed, such as snowdrop lectins (GNA), wheat α -amylase inhibitors (WAI) and bean chitinases (BCH). Insecticidal capability of these transgenic plants was tested in peach-potato aphid (*Myzus persicae*). The best insecticidal effect was recorded for genes that code

lectins (GNA) from snowdrop (*Galanthus nivalis*). GNA which exerted an adverse effect on insect development, impaired their fertility and consequently caused a marked reduction in propagation of insect population (Gatehouse et al., 1996).

In a subsequent study Gatehouse et al. (1997) tested the influence of transgenic potato plants expressing above-mentioned proteins in larvae of moth (*Lacanobia olearacea*), which is not only a significant potato plant pest, but also a vector of viral infections. All the plants tested which were expressing GNA showed an enhanced level of resistance. These results support the hypothesis that GNA has a significant adverse effect on insects.

Despite potato plants containing a lectin gene being resistant to some pests they in contrast became more attractive for others. It seems that alteration of particular physiological processes in potato plants carrying lectin genes is likely to have occurred; that resulted in decreased production of foliar glycoalkaloids, which are substances that cause indigestibility of plants for many mammals and insects (Birch et al., 2002).

In 1999 Ewen and Pusztai (1999) published their study dealing with feeding GNA transgenic potatoes to rats; this led to cell proliferation of their gastric mucosa and alterations of intestinal mucosa. Whereas alterations of gastric mucosa were ascribed to the presence of GNA, proliferation of intestinal mucosa was considered to be associated with genetic transformation. Despite the fact that the professional public highly criticized their opinion and no further study appeared that would confirm a toxic effect of plants as a result of genetic modification in the following years, it elicited reassessment of procedures for food safety testing and formulation of decrees concerning GM foodstuffs (Slater et al., 2003).

2.2. Bacteria, fungi and viruses

2.2.1. Increased resistance to bacteria and fungi

2.2.1.1. Potato carrying a gene for temporin A

The most serious fungal disease of potato plants is potato blight (*Phytophthora infestans*). Temporin A protein producing potato plants display resistance to this disease. Temporin A is a small naturally occurring antimicrobial peptide, which enhanc-

es plant resistance not only to potato blight, but also to wet rot of bacterial origin. This is a disease caused by fungus *P. erythroseptica* and bacterium *Erwinia carotovora*. The results obtained confirm that transgenic potato plants that express temporin A can serve as a good tool for control of most significant fungal pathogens such *P. infestans* and *P. erythroseptica* (Osusky et al., 2004).

2.2.1.2. Potato carrying a gene for glucose oxidase

Another approach to plant protection against pathogens is hydrogen peroxide production. Glucose oxidase gene from *Aspergillus niger* expression was tested from that aspect. In the presence of molecular oxygen, this enzyme catalyses β -D-glucose oxidation with a release of gluconic acid and hydrogen peroxide. Potato plants that produce hydrogen peroxide are characterized by increased resistance against potato blight (*P. infestans*) and to bacterial rot caused by *Erwinia carotovora* (Wu et al., 1995).

2.2.1.3. Potato carrying a gene *ac2*

Fungal infections in potato plants may be likewise controlled by generation of transgenic plants carrying gene *ac2* from amaranth – *Amaranthus caudatus* (Liapkova et al., 2001). Protein resulting from expression of this gene is highly homologous with cystein/glycin rich domains in the chitin binding proteins (Broekaert et al., 1992). These can bind to chitin present in internal fungal cell walls causing alteration of their polarity and inhibition of growth of the fungi (Selitrennikoff, 2001).

2.2.2. Increased resistance to viruses

2.2.2.1. Leafroll luteovirus

A significant viral pathogen of potato plants is luteovirus (*Poleovirus* sp.) spread by aphids; that causes a disease with the signs of potato leafroll luteovirus (PLRV) infection. Resistance of plants against this virus was investigated after insertion of the coat protein gene of PLRV into the genome of potato plants. Although a detectable level of coat protein was not accumulated in any of the tested

plants, virus infected transgenic plants contained markedly lower levels of viral antigen than control plants; this resulted from a reduced rate of virus multiplication in transgenic plants (van der Wilk et al., 1991).

2.3. Herbicides

Besides plants resistant to insect pests and microbial pathogens, potato plants resistant to herbicides have also been generated. Validity of such modifications consists above all in potential application of an herbicide in the most suitable period with concurrent maximum reduction of weeds (Slater et al., 2003). Insertion of the *bar* gene (*PAT*) from bacterium *Streptomyces hygroscopicus* into potato plants is an example; thus modified potato plants are resistant to herbicide phosphinothricin (Padegimas et al., 1994).

2.4. Abiotic factors

Besides biological factors, also factors of environmental life such as ambient temperature, water availability and soil salt level of affect plants. Free radicals and oxidative stress result from the effect of stress factors. Due to continuous thinning of the ozone layer and climatic changes related to global warming, one of the major biotechnological aims is to produce stress resistant plants (Slater et al., 2003).

2.4.1. Potato with resistance to salt in soil

Potato plants resistant to increased soil salt levels were produced by insertion of a gene for glyceraldehyde-3-phosphate-dehydrogenase (GPD) from oyster mushroom (*Pleurotus sajor-caju*) into potato plant genome. The effect of the protein was tested by cultivation of potato plants in sodium chloride containing soil. Whereas GPD-free potato plants died in several days, transgenic plants exhibited a high tolerance to the presence of salts (Jeong et al., 2001).

2.4.2 Potato with resistance to cold and frost

All plants are sensitive to cold and frost caused damage. When the ambient temperature decreases

(-1°C), ice crystals are formed in the extracellular matrix. This causes dehydration of cytoplasm with subsequent shrinkage of membranes. Electrolytes are concurrently released from damaged cells. Potato plants resistant against cold and frost were produced by means of a synthetic gene for AFP protein, derived from Winter flounder (*Pseudopleuronectes americanus*). The generated transgenic plants were more resistant to frost-caused damage, and decreased loss of electrolytes occurring due to low temperatures in comparison with control plants. Resistance to frost directly correlated with the rate of the AFP protein expression in potato plant leaves. Validity of this modification not only consists in the possibility to cultivate commercial crops in different geographical zones, but also to prolong the vegetation season (Wallis et al., 1997).

3. GM potatoes in nutrition

3.1. Potato with increased production of amino acids

Transgenic potato plants containing a gene for non-allergenic protein AmA1 from *A. hypochondriacus* were produced. Tubers of such modified potato plants are in contrast to control plants characterized by increased production of all amino acids. Production of the following amino acids is particularly important: lysine, methionine, cysteine and tyrosine; their levels are highly limited in commonly grown potato plants. This modification may be particularly beneficial for the improvement of human nourishment; its use in the struggle against malnutrition of the poorest children in India (Chakraborty et al., 2000).

3.2. Production of human lactic β -casein

Transgenic potato plants producing human lactic β -casein might also be significant for nourishment. Despite the fact that amount of casein produced by potato plants is relatively low, this experiment indicates that casein can be expressed in edible crops. Human β -casein produced by plants might be used in future for the production of human milk proteins such as lactoferrin and lysozyme or for preparation of baby food with increased nutritional value and preventive effects against gastric and intestinal dysfunctions in children (Chong et al., 1997).

3.3. Flour with improve functional qualities

A paste made of potato flour is characterized by a low elasticity and its use in the food industry is thus limited. Potato plants carrying a gene for low-weight glutenin (LMW-GS-MB1) have been produced with the aim to improve functional qualities of flour. The source of the gene is wheat (*Triticum aestivum* variety Chinese spring) seed containing this protein as one of essential stock proteins. LMW-GS-MB1 units accumulated in transgenic tubers function as a polymer and interconnect them either mutually or with other constituents present in tubers; they consequently form a matrix that leads to a threefold increase in potato flour viscosity (Benmoussa et al., 2004).

3.4. Potato with lower sugar content

Genetically modified potato plants have also been produced in the Czech Republic. These are potatoes with an inserted gene for phosphofructokinase from bacterium *Lactobacillus bulgaricus*. This gene causes degradation of simple sugars via glycolytic pathway. Despite the fact that potato plants contain their own phosphofructokinase, there is one substantial difference. Potato plant enzyme is (in contrast to bacterial enzyme) cold sensitive and consequently stops to functioning at lower temperatures. This causes problems during potato storage at low temperatures as they get sweet due to accumulation of simple sugars. Moreover, potatoes containing higher amounts of simple sugars turn brown during frying and are consequently less attractive for consumers. Transgenic potato plants not only have lower sugar content, but moreover, chips prepared from such potatoes are lighter in colour than those prepared from non-modified ones (Navratil et al., 1998).

4. Production of vaccines and human proteins

Humans have been using plants for medical purposes for several thousand years; however production of biopharmaceuticals from plants with the assistance of gene engineering was introduced much later. Production of recombinant plant proteins offers a series of potential benefits such as

- (i) cheap production and storage,
- (ii) large-scale manufacture of biopharmaceuticals,
- (iii) higher health safety level in comparison with vaccines of animal origin.

Further advantage of plant biopharmaceuticals is potential elimination of a purification process, in case plant tissues are used as food. Last but not least, it is noteworthy that the target protein may be inserted into a particular cell compartment (chloroplast); its stability is consequently increased or this protein may even be expressed in this compartment (Daniell et al., 2001). On the other hand, technology of plant bioreactors is limited by the following factors:

- (i) low protein yield usually caused by its low stability,
- (ii) problems during protein production that are reflected in variable quality of the final product,
- (iii) even minor differences in post-translational modifications can potentially affect the activity and/or immunogenicity of the recombinant molecules (Miele, 1997).

Despite the majority of recombinant proteins being produced by microorganisms, transgenic plants represent an alternative system for their production. The most suitable plant for production of human vaccines (edible vaccines) seems to be the banana plant for the following reasons: it is consumed easily by children, consumption is in raw form and it occurs naturally in developing countries. However, tomato plants, maize and potato plants are rather used as model systems in typical experiments dealing with expression of foreign proteins in plants (Stirn and Lorz, 2003).

4.1. Production of vaccines against viral and bacterial diseases

4.1.1. Vaccines against viral diseases of man and animals

4.1.1.1. Viral diseases of humans

4.1.1.1.1. Vaccine against hepatitis B

Vaccines against hepatitis B classified as subunit vaccines are based on the principle of HBsAg expression in yeasts. Despite this vaccine being intended for parenteral administration, there is no obstacle for oral use. Oral vaccines aimed at immunization can be administered repeatedly and easily, the immune

response on mucosal sites increases and they also stimulate humoral immunity. Therefore, transgenic potato plants carrying a gene for surface antigen of hepatitis B – HBsAg have been generated (Kong et al., 2001).

The capability of “potato plant antigen” to induce immune response after oral administration was tested in mice. Experiments showed that primary immune response was stimulated in animals given raw potatoes carrying HBsAg. Administration of one parenteral injection with HBsAg at the time of decreased primary response produced a rapid and strongly expressed immune response in experimental animals. That persisted for at least five months. It was concurrently detected that immunogenicity of vaccines is reduced in cooked potatoes which makes the use of these plants as “edible vaccines” for people impossible (Kong et al., 2001).

4.1.1.1.2. Vaccine against Norwalk virus

Transgenic potatoes carrying a gene for the capsid protein of Norwalk virus – NVCP (causative agent of epidemic gastroenteritis of humans) have been prepared. Capsid protein was expressed in potato tubers, in the amount of approximately 0.37% of total protein. Immunogenicity of transgenic potato plants was tested in mice; IgG antibodies against recombinant Norwalk virus were detected in them (Mason et al., 1996). Capability of this “edible vaccine” to activate the immune system was tested also in human volunteers; immune response was activated in the majority (95%) of the people (Tacket et al., 2000).

4.1.1.1.3. Vaccine against papillomaviruses

Human papillomaviruses (HPV) are involved in the development of cervical cancer. Because this diagnosis is relatively frequent in women, development of a preventative vaccine against this disease would be beneficial. The purpose of generation of transgenic potato plants carrying the main structural protein of L1 HVP virus (type HVP-16) was protein expression into VLP particles utilizable in prophylactic vaccination. Oral administration of “L1 tubers” to mice led to formation of a weak immune response, however, in several animals only. Despite oral administration of plant transgenic ma-

terial induced a protective immune response in this case, the immune response was not sufficient due to a low protein yield (Biemelt et al., 2003).

4.1.1.1.4. Vaccine against hantaviruses

Serious diseases in humans may be also caused by hantaviruses (*Bunyaviridae*) transmitted by rodents. Humans can get infected by a direct contact with a diseased animal or by inhalation of contaminated urine, faeces or saliva. The disease particularly affects kidneys and lungs; further signs are hemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome (Murray et al., 2002). A nucleocapsid protein (S-segment) from Puumala virus, the causative agent of benign disease *nefrophia epidemica* was selected for generation of transgenic potato plants. Capability of the hantavirus protein to stimulate an immune response was tested in rabbits that were intramuscularly and intraperitoneally inoculated with a leaf extract. Due to a stable expression of nucleocapsid protein in potato tubers, recombinant hantavirus protein can be used not only for preparation of alternative vaccines, but also for development of novel diagnostic systems and for basic research (Kehm et al., 2001).

4.1.1.2. Viral diseases of animals

4.1.1.2.1. Vaccine against lethal rabbit hemorrhagic disease (RHDV)

A virus causing lethal contagious rabbit hemorrhagic disease (RHDV) is alike Norwalk virus and is classified as a calcivirus. Hemorrhagic syndrome and acute kidney damage are characteristic of the infection; mortality of adult animals is high. The primary structural protein of this virus is VP60 protein, which was used for transformation of leaf explants from potato plants. Despite the animals immunized with the leaf extract containing protein VP60 exhibited high titres of antibodies, the use of potato tubers for oral immunization of rabbits did not appear to be effective enough because of a low expression of the protein (Castanon et al., 1999). Oral immunization of rabbits with transgenic potatoes producing VP60 protein only confirmed the above mentioned hypothesis (Martin-Alonso et al., 2003).

4.1.1.2.2. Vaccine against infectious bronchitis virus (IBV)

Furthermore, expression of S1 glycoprotein of the infectious bronchitis virus (IBV) in potato plants was investigated; subsequently, immunogenicity in mice and chickens was studied. Infectious bronchitis virus is classified as coronavirus and is the causative agent of a highly contagious disease of respiratory, excretory and urogenital tracts, characterized by high mortality in the affected herds. The gene for S1 glycoprotein was cloned into *Escherichia coli* and introduced into potato plants using *Agrobacterium tumefaciens*-mediated transformation. The extract from potato tubers containing S1 protein was given to mice. Virus neutralizing antibodies were detected in those mice after administration of three doses of the extract. Similar results were recorded in chickens and moreover, they were completely protected against IBV virus after the third vaccination with transgenic potatoes (Zhou et al., 2003).

The same research team (Zhou et al., 2004) was the first to prepare the transgenic plant expressing the full-length S protein of IBV. The transgenic plants were used for oral and intramuscular immunization of chickens. The results demonstrated a high titer of anti-IBV antibodies, which protected the experimental animals from the infection of the virulent IBV in challenge. The results show the possibility of transgenic potato plants expressing S protein of IBV to be used in the infectious bronchitis control.

4.1.1.2.3. Vaccine against rotaviruses

Rotavirus capsid protein VP6 was inserted into potato plants. Rotaviruses represent a large group of viruses causing gastroenteritis in various species of mammals and birds. They are also the most common causative agents of diarrhoeal diseases in children worldwide (Murray et al., 2002). The major structural protein of the virus is VP6 protein; that was also used for potato plant transformation. The first study of plant transformation by means of the VP6 virus was focused on the bovine rotavirus A expression. After an injection of the potato extract into mice used for testing immunogenicity of recombinant protein, antibodies against VP6 protein were detected (Matsumura et al., 2002). Mouse rotavirus was used as a source of VP6 protein in an-

other study. Protein effectivity was again tested in mice. These were fed with transgenic potato tubers as a source of the protein. Oral immunization of mice stimulated production of IgG and IgA antibodies against capsid protein and thus represented a progress in the development of rotavirus vaccine by means of agricultural plants (Yu and Langridge, 2003).

4.1.1.2.4. Vaccine against infectious gastroenteritis (TGEV)

Coronavirus that causes infectious gastroenteritis (TGEV) may also be a causative agent of diarrhoeal disease. This virus particularly affects newborn piglets and causes a high mortality in herds. It is most suitable to use viral glycoprotein S (gS) for the production of vaccines due to its immunogenicity and resistance to degradation in the intestines. That was also confirmed in mice, intraperitoneally and orally given an extract from transgenic potatoes. Antibodies against TGEV were detected in mouse blood sera in both the groups. Since no immunotolerance was established in any of the vaccinated animals, the feasibility of vaccinating farm animals is indicated through the diet in the future (Gomez et al., 2000).

4.1.1.2.5. Vaccine against foot and mouth disease

Transgenic potato plants carrying a gene for structural VP1 protein of foot and mouth disease virus, the agent causing an economically important disease affecting meat-producing animals, was similarly produced. As in the previous studies, antibodies against viral protein were detected in the immunized animals (mice) and resistance to experimental infection with foot and mouth disease was found (Carrillo et al., 2001).

4.1.2. Vaccines against bacterial diseases of man and animals

4.1.2.1. Vaccine against enterotoxigenic strains of *E. coli* (ETEC)

A thermolabile enterotoxin B (LT-B) from bacteria *E. coli* was expressed in potato plants. The

toxin is produced by enterotoxigenic strains of *E. coli* (ETEC) that colonize small intestine and cause acute watery diarrhoea. Faecal IgA and serum anti-LT-B IgG antibodies were detected in mice that had been given three doses of transgenic potatoes. Despite the infection in mice was induced, full immunity against the bacterial disease was not developed in this case (Mason et al., 1998). The mentioned modification was also investigated in another study, which was focused on testing capability of recombinant LT-B protein to produce mucosal and total systemic antibody response in mice. Mice used in the experiment were either pre-immunized or non-immunized. Whilst no anti-LT antibody formation was detected in the non-immunized mice, anti-LT IgA antibodies were produced by the immunized mice. A higher antibody response was found after LT-B-protein administration through intestinal intubation than after oral administration (Lauterslager et al., 2001).

4.1.2.2. Vaccine against of toxin *Vibrio cholerae*

Other potato plants produce CTB protein, a non-toxic component of cholera toxin B (*Vibrio cholerae*). After transformation of plants, CTB protein was detected in potato plant leaves and tubers. The amount of the protein was approximately 0.3% of total plant protein. The CTB protein accumulated in potato plant tissues is capable of oligomer formation, whilst its natural biochemical and immunological qualities are maintained. This advantage can be utilised in production of oligomer CTB protein by plants with the purpose to induce formation of antibodies against cholera toxin in humans and thus to provide sufficient immunity against this disease (Arakawa et al., 1997).

4.2. Production of human proteins

Besides vaccines, plants may serve as bioreactors for the production of immune system substances and protective substances present in milk such as human lactoferrin. However, in this case, target protein yield is a limiting factor. Generally, the amount of the protein produced by plants is less than 1% of total soluble protein. The yields of human protein encoding gene expression are all lower (Daniell et al., 2001).

4.2.1. Production of human lactoferrin

One of human proteins inserted to the genome of potato plants in the same way was human lactoferrin. It is a glycoprotein present above all in maternal milk and less in tears and bile. Lactoferrin has a protective function because it can bind iron, which makes it inaccessible for bacteria. The potential for lactoferrin to act both as an antimicrobial and an immune regulatory agent in addition to its nutritional and pharmaceutical value has led to development of transgenic potato plants carrying a cDNA fragment encoding human lactoferrin (hLF). Biological qualities of lactoferrin, formed by transgenic potatoes were confirmed in four different human pathogenic bacterial strains. GM potato plants were shown to be able to produce human lactoferrin that maintains biological, bacteriostatic and bactericidal qualities against various pathogenic bacteria. Moreover expression of human milk proteins such as lactoferrin or β -casein in potato opens the way for addition of a variety of plant-synthesized hypoallergenic human milk proteins to infant formulas and baby foods (Chong and Langridge, 2000).

4.2.2. Production of human interferons (HuIFN- α -2b and HuIFN- α -8)

Further genetic modification was the insertion of the gene for human interferon- α -2b (HuIFN- α -2b) and α -8 (HuIFN- α -8) into the genome of potato plants. Interferons are classified as antiviral cytokines responsible for various cytotoxic effects including anti-tumour activity. The HuIFN- α genes introduced into the potato plant was correctly translated and transcribed in plant cells and their biological activity was verified by inhibition of vesicular stomatitis virus (VSV) replication on a human amniotic cell line. It is supposed that potato plants carrying genes for human interferons will be used as food additives or as additional substances for treatment of infectious diseases and decreased immunity (Ohya et al., 2001).

4.2.3. Production of human tumour necrotizing factor (HuTNF- α)

Ohya et al. (2002) produced transgenic potato plants carrying a gene for human tumour necro-

tizing factor- α (HuTNF- α). This cytokinin is produced by stimulated cells of the immune system and can improve inflammatory immune response of the organism or cause *in vitro* lysis of tumour cells. Transgenic potato plants were shown to produce HuTNF- α , and the extract from transformed plants causes a cytotoxic effect. This capability together with a relatively high protein yield will predestine transgenic plants producing TNF- α to be used in human and veterinary medicine (Ohya et al., 2002).

Oral vaccines are potentially usable for production of any vaccine, which comprises or contains subunit components. Oral administration of subunit vaccines is particularly suitable for stimulation of immunity against the pathogens that enter the body via intestines. Oral vaccines are also important for the control of pathogenic infections of other mucosal surfaces (hepatitis B, HIV) due to the shared origin of the mucosal immune system. As plants can produce large amounts of subunit vaccines, they may be particularly used for the control of diseases affecting large populations of people. Production at low costs, stability of plant vaccines during storage at ambient temperature, reduction of material and nursing staff (administration of injections) predestines edible vaccines to be particularly used in developing countries (Streatfield and Howard, 2003).

5. Other types of genetic modifications of potato plants

Genetic engineering extends the potential use of natural plant materials, e.g. in the production of modified starch or synthesis of novel polymers. Plant biomaterials, characterized by their renewability and biodegradability, might replace synthetic plastic materials and elastomers made of petroleum in future (Moire et al., 2003).

5.1. Production of biopolymers

5.1.1. Production of polyhydroxyalkanoates

Polyhydroxyalkanoates, polyesters of hydroxyacids formed by long monomers are naturally synthesized by various strains of bacteria. Their qualities are identical with those of elastomers and adhesive materials, and therefore they can be used for gen-

eration of a broad range of products (Moire et al., 2003). Transgenic potato plants carrying the gene *phaC1* from soil bacterium *Pseudomonas oleovorans* were made with the aim to produce polyhydroxyalkanoates. This gene encodes enzyme Pha-C1 polymerase that can transform 3-(R)-hydroxyacetic acid to an inert polymer. Due to the fact that potato plants in contrast to bacteria do not contain any enzyme able to cause degradation of this polymer, it could be detected in plant cell culture (Romano et al., 2003).

5.2. Production of spiderweb in transgenic potato

It is also noteworthy that spiderweb fibre can be produced in potato plants. Scheller et al. (2001) made a fusion protein (FA2) composed of a synthetic *MaSP1* gene (spidroin) of spider of *Nephila clavipes* species and a gene for fibroin from larvae of silkworm moth (*Bombyx mori*). Synthetic hybrid protein was detected in potato plant leaves and tubers, which constituted at the most 2% of total soluble protein. Spider protein has very good mechanical qualities, which might be used in human and veterinary medicine or in the industry for production of foils and various fibres. Plant bioreactors might become an alternative solution to the production of materials made of petroleum sources that are gradually depleted.

5.3. Production of a freeze-thaw-stable potato starch

Potatoes containing stable starch after freezing and thawing may be noteworthy for consumers. If potato-based commodities are frozen and subsequently thawed, their texture changes due to repeated degradation of the glucan chains. This effect may be suppressed, but only with the use of chemical compounds. Genetic engineering can change the structure and composition of starch and thus generate starch composed of short amylopectin chains that do not contain amylase. This modification is primarily beneficial to the life environment and consumers (elimination of chemical processes); decreased energy consumption due to a lower temperature necessary for starch cooking is equally important (Jobling et al., 2002).

5.4. Increased synthesis of lipids in potato tubers

Besides practical use, creating transgenic potato plants represents a good tool for investigation of enzymatic pathways in various parts of plant bodies such as clarification of factors limiting lipid synthesis in potato tubers. These contain high levels of starch; however, they are scarce in lipids. High levels of the enzyme acetyl-CoA carboxylase are produced by transgenic potato plants and consequently, synthesis of fatty acids and triglycerides is increased. It follows from the above results that potato tubers may function as storage organs of lipids (Klaus et al., 2004).

6. REFERENCES

- Arakawa T., Chong D.K., Merritt J.L., Langridge W.H. (1997): Expression of cholera toxin B subunit oligomers in transgenic potato plants. *Transgenic Research*, 6, 403–413.
- Benmoussa M., Vezina L.P., Page M., Gelinat P., Yelle S., Laberge S. (2004): Potato flour viscosity improvement is associated with the expression of a wheat LMW-glutenin gene. *Biotechnology and Bioengineering*, 87, 495–500.
- Biemelt S., Sonnewald U., Galmbacher P., Willmitzer L., Müller M. (2003): Production of human papillomavirus type 16 virus-like particles in transgenic plants. *Journal of Virology*, 77, 9211–9220.
- Birch A., Geoghegan I.E., Griffiths D.W., McNicol J.W. (2002): The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solanum tuberosum*). *Annals Applied Biology*, 140, 143–149.
- Broekaert W.F., Marien W., Terras F.R., De Bolle M.F., Proost P., Van Damme J., Dillen L., Claeys M., Rees S.B., Vanderleyden J. (1992): Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochemistry*, 31, 4308–4314.
- Carrillo C., Wigdorovitz A., Trono K., Dus Santos M.J., Castanon S., Sadir A.M., Ordas R., Escribano J.M., Borca M.V. (2001): Induction of a virus-specific antibody response to foot and mouth disease virus using the structural protein VP1 expressed in transgenic potato plants. *Viral Immunology*, 14, 49–57.
- Castanon S., Marin M.S., Martin-Alonso J.M., Boga J.A., Casais R., Humara J.M., Ordas R.J., Parra F. (1999): Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. *Journal of Virology*, 73, 4452–4455.
- Chakraborty S., Chakraborty N., Datta A. (2000): Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from *Amaranthus hypochondriacus*. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 3724–3729.
- Chong D.K., Langridge W.H. (2000): Expression of full-length bioactive antimicrobial human lactoferrin in potato plants. *Transgenic Research*, 9, 71–78.
- Chong D.K., Roberts W., Arakawa T., Illes K., Bagi G., Slattery C.W., Langridge W.H. (1997): Expression of the human milk protein beta-casein in transgenic potato plants. *Transgenic Research*, 6, 289–296.
- Daniell H., Streatfield S.J., Wycoff K. (2001): Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends in Plant Science*, 6, 219–226.
- Davies H.V. (1996): Recent developments in our knowledge of potato transgenic biology. *Potato Research*, 39, 411–427.
- Ewen S.W.B., Pusztai A. (1999): Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet*, 354, 1353–1354.
- Gatehouse A.M.R., Down R.E., Powell K.S., Sauvion N., Rahbe Y., Newell Ch.A., Merryweather A., Hamilton W.D.O., Gatehouse J.A. (1996): Transgenic potato plants with enhanced resistance to the peach-potato aphid *Myzus persicae*. *Entomologia Experimentalis et Applicata*, 79, 295–307.
- Gatehouse A.M.R., Davison G.M., Newell C.A., Merryweather A., Hamilton W.D.O., Burgess E.P.J., Gilbert R.J.C., Gatehouse J.A. (1997): Transgenic potato plants with enhanced resistance to the tomato moth, *Lacania oleracea*: Growth room trials. *Molecular Breeding*, 3, 49–63.
- Gomez N., Wigdorovitz A., Castanon S., Gil F., Ordas R., Borca M.V., Escribano J.M. (2000): Oral immunogenicity of the plant derived spike protein from swine-transmissible gastroenteritis coronavirus. *Archives of Virology*, 145, 1725–1732.
- Jeong M.J., Park S.C., Byun M.O. (2001): Improvement of salt tolerance in transgenic potato plants by glyceraldehyde-3 phosphate dehydrogenase gene transfer. *Molecules and Cells*, 12, 185–189.
- Jobling S.A., Wescott R.J., Jeffcoat R., Schwall G.P. (2002): Production of a freeze-thaw-stable potato starch by antisense inhibition of three starch synthase genes. *Nature Biotechnology*, 20, 295–299.
- Kehm R., Jakob N.J., Welzel T.M., Tobiasch E., Viczian O., Jock S., Geider K., Sule S., Darai G. (2001): Expres-

- sion of immunogenic Puumala virus nucleocapsid protein in transgenic tobacco and potato plants. *Virus Genes*, 22, 73–83.
- Klaus D., Ohlrogge J.B., Neuhaus H.E., Dormann P. (2004): Increased fatty acid production in potato by engineering of acetyl-CoA carboxylase. *Planta*, 219, 389–396.
- Kong Q., Richter L., Yang Y.F., Arntzen C.J., Mason H.S., Thanavala Y. (2001): Oral immunization with hepatitis B surface antigen expressed in transgenic plants. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 11539–11544.
- Lauterslager T.G., Florack D.E., van der Wal T.J., Moltthoff J.W., Langeveld J.P., Bosch D., Boersma W.J., Hilgers L.A. (2001): Oral immunisation of naive and primed animals with transgenic potato tubers expressing LT-B. *Vaccine*, 19, 2749–2755.
- Liapkova N.S., Loskutova N.A., Maisurian A.N., Mazin V.V., Korableva N.P., Platonova T.A., Ladyzhenskaia E.P., Evsyunina A.S. (2001): Isolation of genetically modified potato plant containing the gene of defensive peptide from *Amaranthus* (in Russia). *Applied Biochemistry and Microbiology*, 37, 349–354.
- Martin-Alonso J.M., Castanon S., Alonso P., Parra F., Ordas R. (2003): Oral immunization using tuber extracts from transgenic potato plants expressing rabbit hemorrhagic disease virus capsid protein. *Transgenic Research*, 12, 127–130.
- Mason H.S., Ball J.M., Shi J.J., Jiang X., Estes M.K., Arntzen C.J. (1996): Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 5335–5340.
- Mason H.S., Haq T.A., Clements J.D., Arntzen C.J. (1998): Edible vaccine protects mice against *Escherichia coli* heat-labile enterotoxin (LT): potatoes expressing a synthetic LT-B gene. *Vaccine*, 16, 1336–1343.
- Matsumura T., Itchoda N., Tsunemitsu H. (2002): Production of immunogenic VP6 protein of bovine group A rotavirus in transgenic potato plants. *Archives of Virology*, 147, 1263–1270.
- Miele L. (1997): Plants as bioreactors for biopharmaceuticals: regulatory considerations. *Trends in Biotechnology*, 15, 45–50.
- Moire L., Rezzonico E., Poirier Y. (2003): Synthesis of novel biomaterials in plants. *Journal of Plant Physiology*, 160, 831–839.
- Murray P.R., Rosenthal K.S., Kobayashi G.S., Pfaller M.A. (2002): *Virology*. In: *Medical Microbiology*. 4th ed. Mosby Inc., An Affiliate of Elsevier Science, St. Louis, Missouri. 427–625.
- Navratil O., Vojtechova M., Fischer L., Blafkova J., Linhart M. (1998): Characterization of transgenic potato plants with an additional bacterial gene coding for phosphofructokinase. *Chemical Papers*, 52, 598–598.
- Ohya, K., Matsumura, T., Ohashi, K., Onuma, M., Sugimoto, C. (2001): Expression of two subtypes of human IFN-alpha in transgenic potato plants. *Journal of Interferon and Cytokine Research*, 21, 595–602.
- Ohya K., Itchoda N., Ohashi K., Onuma M., Sugimoto C., Matsumura T. (2002): Expression of biologically active human tumor necrosis factor-alpha in transgenic potato plant. *Journal of Interferon and Cytokine Research*, 22, 371–378.
- Osusky M., Osuska L., Hancock R.E., Kay W.W., Misra S. (2004): Transgenic potatoes expressing a novel cationic peptide are resistant to late blight and pink rot. *Transgenic Research*, 13, 181–190.
- Padegimas L., Shul'ga, O.A., Skriabin, K.G. (1994): Creation of transgenic plants *Nicotiana tabacum* and *Solanum tuberosum*, resistant to the herbicide phosphinothricin (in Russian). *Molecular Biology*, 28, 437–443.
- Perlak F.J., Stone T.B., Muskopf Y.M., Petersen L.J., Parker G.B., McPherson S.A., Wyman J., Love S., Reed G., Biever D., Fischhoff D.A. (1993): Genetically improved potatoes: protection from damage by Colorado potato beetles. *Plant Molecular Biology*, 22, 313–321.
- Romano A., Vreugdenhil D., Jamar D., van der Plas L.H.W., de Roo G., Witholt B., Eggink G., Mooibroek H. (2003): Evidence of medium-chain-length polyhydroxyoctanoate accumulation in transgenic potato lines expressing the *Pseudomonas oleovorans* Pha-C1 polymerase in the cytoplasm. *Biochemical Engineering Journal*, 16, 135–143.
- Scheller J., Guhrs K.H., Grosse F., Conrad U. (2001): Production of spider silk proteins in tobacco and potato. *Nature Biotechnology*, 19, 573–577.
- Selitre C.P. (2001): Antifungal Proteins. Review. *Applied and Environmental Microbiology*, 67, 2883–2894.
- Slater A., Scott N.W., Fowler M.R. (eds.) (2003): *Plant Biotechnology, the Genetic Manipulation of Plants*. 1st ed. Oxford University Press Inc., New York, USA. 346 pp.
- Stirn S., Lorz H. (2003): Genetically modified plants, 26–61. In: Heller K.J. (ed.): *Genetically Engineered Food*. Wiley-Vch GmbH & Co. KGaA, Weinheim. 276 pp.
- Streatfield S.J., Howard J.A. (2003): Plant-based vaccines. *International Journal of Parasitology*, 33, 479–493.
- Tacket C.O., Mason H.S., Losonsky G., Estes M.K., Levine M.M., Arntzen C.J. (2000): Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *Journal of Infectious Diseases*, 182, 302–305.

- Wallis J.G., Wang H., Guerra D.J. (1997): Expression of a synthetic antifreeze protein in potato reduces electrolyte release at freezing temperatures. *Plant Molecular Biology*, 35, 323–330.
- van der Wilk F., Posthumus-Lutke Willink D., Huisman M.J., Huttinga H., Goldbach R. (1991): Expression of the potato leafroll luteovirus coat protein gene in transgenic potato plants inhibits viral infection. *Plant Molecular Biology*, 17, 431–439.
- Wu G., Shortt B.J., Lawrence E.B., Levine E.B., Fitzsimmons K.C., Shah D.M. (1995): Disease resistance conferred by expression of a gene encoding H₂O₂-generating glucose oxidase in transgenic potato plants. *Plant Cell*, 7, 1357–1368.
- Yu J., Langridge W. (2003): Expression of rotavirus capsid protein VP6 in transgenic potato and its oral immunogenicity in mice. *Transgenic Research*, 12, 163–169.
- Zhou J.Y., Wu J.X., Cheng L.Q., Zheng X.J., Gong H., Shang S.B., Zhou E.M. (2003): Expression of immunogenic S1 glycoprotein of infectious bronchitis virus in transgenic potatoes. *Journal of Virology*, 77, 9090–9093.
- Zhou J.Y., Cheng L.Q., Zheng X.J., Wu J.X., Shang S.B., Wang J.Y., Chen J.G. (2004): Generation of the transgenic potato expressing full-length spike protein of infectious bronchitis virus. *Journal of Biotechnology*, 111, 121–130.

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Corresponding Author:

Prof. MVDr. Ivo Pavlik CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 601, fax +420 541 211 229, e-mail: pavlik@vri.cz
