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Transgenic Potato Plants with Overexpression of Dihydroflavonol Reductase Can Serve as Efficient Nutrition Sources

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ABSTRACT: Potato (*Solanum tuberosum*) is considered to be one of the most important crops cultivated in Europe and the entire world. The tubers of the potato are characterized by rich starch and protein contents and high concentrations of antioxidants, such as vitamin C and flavonoids. Notably, the presence of the phenolic antioxidants is of high importance as they have health-related properties. They are known to reduce the incidence of atherosclerosis, prevent certain kinds of cancer, and aid with many other kinds of diseases. The aim of this study was to find the most efficient way to increase the content of phenolic antioxidants in potato tubers through transgenesis. The results showed that the most efficacious way to achieve this goal was the overexpression of the dihydroflavonol reductase gene (DFR). The produced transgenic potato plants served as a nutrition source for laboratory rats; the study has confirmed their nontoxicity and nutritional benefits on the tested animals.

KEYWORDS: *transgenic potato, flavonoids, rat feeding, antioxidants*

INTRODUCTION

The most essential crop in Europe, besides cereals, is potatoes. They have expanded all over the world, reaching eastern Asia and India, becoming the third most widely consumed plant product by humans after wheat and rice. Potato tubers are valued for their high starch content (up to 30.4% of their fresh weight) and digestibility.[1] The proteins, the content of which may be as high as 2%, are very valuable for their amino acid composition. Potato tubers' biological value (BV) may be anywhere between 90 and 100: compare eggs (100), soybeans (84), and beans (73).[2] Furthermore, potatoes are a rich source of vitamins and minerals, such as vitamin C (0.20 mg g^{-1} FW), vitamin B6 (2.5 μg g^{-1} FW), potassium (5.64 mg g^{-1} FW), phosphorus (0.30−0.60 mg g^{-1} FW), and calcium (0.06−0.18 mg g^{-1} FW).[2,3] The health-oriented character of the potato is, among other factors, a consequence of its high content of antioxidants (resulting from its high content of vitamin C) and from the presence of antioxidant phenolic compounds, such as phenolic acids and flavonoids.[4] Phenolics are produced in plants through the phenylpropanoid pathway. Flavonoid biosynthesis depends on several crucial enzymes, such as chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol reductase (DFR). The simultaneous action of these three enzymes allows the plant to produce anthocyanidins and, after glycosylation by proper glucosyltransferases (GT), anthocyanins.[5] The content of phenolic acids ranges from 0.6 to 2.7 mg g^{-1} FW, with chlorogenic acid, neochlorogenic acid, and caffeic acid being the most abundant.[6] Petunidin and pelargonidin derivatives are the most abundant anthocyanidin compounds found in the potato,[7] and their levels reach 0.5 and 0.05 mg g^{-1} DW, respectively.

The phenolic compounds are of highest importance in protecting plants against biotic and abiotic threats. Pathogenic attacks and environmental stresses, two of the largest problems in crop cultivation, result in the reduction of plant productivity. The most frequent diseases of the potato are bacterial wilt caused by *Ralstonia solanacearum*, bacterial soft rot caused by *Erwinia carotovora*, and the late blight and early blight caused by the fungi *Phytophthora infestans* and *Alternaria solani*. It was proven that a large group of phenylpropanoids constitute the most important compounds in protecting maize and sorghum plants against pathogens.[8,9] Poiatti *et al.*[10] showed that the level of total phenolics and flavonoids correlates with the potato plant's resistance to pathogen attack.

It has been proven that diets rich in phenolic compounds have a positive effect on health, reducing the incidence of atherosclerotic heart disease, certain cancers, and macular degeneration and mitigating the severity of cataracts.[11,12] When rats were fed a darker purple potato, the serum antioxidant capacity was higher as compared to the control diet. Furthermore, in the related studies, antioxidant enzyme activities in the liver of those rats were significantly greater than in the control group.[13] High levels of dietary antioxidants may enhance protection against oxidative damage in an organism. Most importantly, phenolic compounds were shown to maintain their stability after industrial potato processing.[7]

The aim of this study was to present an efficient way to achieve the highest phenolic antioxidant level in potato by means of genetic engineering and to test whether the new potatoes may serve as a healthier source of nutrition.

MATERIALS AND METHODS

Plant Material. Potato plants (*Solanum tuberosum* L. cv. Desiree) were obtained from Saatzucht Fritz Lange KG (Bad Schwartau, Germany). Control and transgenic plants were grown in soil under a 16 h light (22 °C) and 8 h darkness (16 °C) regimen in a greenhouse.

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Plants were grown in individual pots and were watered daily. Tubers were harvested after 4 months of growth and subsequently used for a field trial conducted near Wroclaw. For biochemical analysis field-grown potatoes were used.

Generation of Transgenic Plants. In this study, five types of transgenic plants were used: type CHS, overexpressing the barley cDNA encoding chalcone synthase (EMBL/GenBank database accession no. X58339); CHI, overexpressing the *Petunia hybrida* cDNA encoding chalcone isomerase (EMBL/GenBank database accession no. X14589); DFR, with overexpression of the *P. hybrida* cDNA encoding dihydroflavonol 4-reductase (EMBL/GenBank database accession no. X15537); type W95, overexpressing CHI and DFR; and finally type W92, overexpressing all three barley and petunia cDNAs. Petunia cDNAs and barley cDNA were provided by Dr. I. Somssich and Dr. H. Hesse, respectively. For leaf explant transformations the pBin vector containing respective cDNA in sense orientation under the control of the 35S promoter and OCS terminator was used. Control (Desi) plants were transformed with an empty vector. The vector was introduced into the *Agrobacterium tumefaciens* strain C58C1:pGV2260 as described before,[14] and the integrity of the plasmid was verified by restriction enzyme analysis. Young leaves of wild-type potato *S. tuberosum* L. cv. Desiree were transformed with *A. tumefaciens* by immersing the leaf explants in the bacterial suspension. Leaf explants inoculated with *A. tumefaciens* were subsequently transferred to callus induction medium followed by shoot regeneration medium.[14] The selection marker was the neomycin phosphotransferase gene (npt II). The transgenic plants were preselected by PCR using primers specific for the kanamycin resistance gene and then selected by means of Northern blot analysis.[15] Total RNA was prepared from frozen young plant leaves using the guanidinium hydrochloride method as described before.[16] Following electrophoresis (1.5% (w/v) agarose, 15% (v/v) formaldehyde), RNA was transferred to nylon membranes (Hybond N, Amersham, UK). Membranes were hybridized overnight at 42 °C with radioactively labeled respective cDNA (CHS, CHI, and DFR) as probes. For W92 plants, DFR cDNA, and for W95, CHI cDNA, were used. Filters were washed three times in 0.1× SSC and 0.1% SDS for 30 min at 65 °C.

Extraction and HPLC Analysis of Phenolic Compounds from Tubers of Transgenic Potato. Tubers were dried and ground, and a weighed amount of 150 mg was extracted with 1 mL of 0.1% HCl in methanol solution in an ultrasonic bath for 15 min. The samples were centrifuged, and the supernatant was transferred to a fresh tube after filtration through a Millipore (0.2 μm) filter. The filtrate was subsequently dried in a Speedvac. The dried extract was resuspended in 1 mL of ultrapure water. The solution was then applied onto the SPE column (Merck). The phenolic compounds were eluted from the solid phase with 40% methanol and analyzed on an HPLC system (Knauer) equipped with an automated sample injector and an UV detector (Knauer variable wavelength monitor type 87.00) connected to a personal computer (HPLC software/hardware package version 2.21A). A LiChroCART 250-4 100 RP-18 (5 μm) column preceded by a LiChroCART 4-4 100 RP-18 (5 μm) precolumn (Merck) was used for sample separation. Compound detection was carried out by on-column measurement of UV absorption at 325 nm. The sensitivity was 0.04 aufs, and the flow rate was 1.0 mL min^{-1}. Phenolics were separated using a mobile phase consisting of the following components: (A) acetonitrile/1% formic acid (90:10 v/v) (Merck); and (B) water/1% formic acid (90:10 v/v). For the first 2 min, isocratic elution was carried out using 10% A in B. From 2 to 25 min, a linear gradient was applied using 10−30% A in B. From 25 to 27 min, a linear gradient was applied using 30−70% A in B. The sample volume was 20 μL. Solvent solutions were vacuum degassed with sonication prior to usage. The calibration graphs of phenolic compounds were prepared by measuring the peak area, and they were linear in the examined range (0.02−0.10 mg mL^{-1}). All experiments were performed at room temperature (20 °C). The compounds were identified and determined on the basis of analysis of standards as described.[17] Pelargonidin 3-rut-5-glu and petunidin 3-rut-5-glu, both acylated with *p*-coumaric acid, were used as standards.

Antioxidant Activity of Transgenic Potato Tuber Extracts. An amount of 150 mg of potato tuber was extracted with 0.1% HCl in methanol solution. The extract was diluted in the range from 2500 to 35000 times with water and directly analyzed using a chemiluminescence method.[18] The experiments were performed in a final volume of 250 μL on white microplates in a freshly prepared solution containing 0.1 M Tris-HCl buffer (pH 9.0) and 4 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). The luminol solution (100 μM) and diluted extracts were automatically injected. The photons produced in the reaction were counted on an EG&G Berthold LB96P microplate luminometer at 30 °C. The antioxidant potential was defined as the amount of tuber extract that inhibits luminal chemiluminescence by 50% and was expressed as IC_{50}.

Densitometry Analysis. Densitometric analysis was performed on the basis of Northern blot analysis, using Vision Works LS v. 6.8 software from UVP (USA). The blots were normalized to 18S ribosomal subunit RNA.

Determination of Starch and Protein Contents in Tubers. For starch measurement, extracted plant material was homogenized in 0.2 M KOH and, following incubation at 95 °C, adjusted to pH 5.5 with 1 M acetic acid. Starch was hydrolyzed with amyloglucosidase, and the released glucose was determined enzymatically.

The crude protein content in samples of tubers was determined according to the standard Kjeldahl procedure (AOAC, 1995) in a Kjeldahl apparatus type K-424/K-314 (Buchi, Germany). At least three measurements were performed for each sample.

Extraction of Steroid Alkaloid Glycosides from Tubers of Transgenic Potatoes. Samples of lyophilized tubers (150 mg) were homogenized, and an internal standard, genistein 7-*O*-glucoside, was applied to each one, to a final concentration of 300 ng/mL. Genistein 7-*O*-glucoside was isolated from *Lupinus luteus* and characterized.[19] Tissue samples were extracted with 10 mL of 80% methanol containing 1% HCl in an ultrasonic bath for 30 min. The suspension was filtered through a Büchner funnel and concentrated under vacuum at 40 °C to 1 mL volume. The extract samples were transferred to the screw-capped vials evaporated to dryness in a stream of nitrogen. The dried samples were stored in a freezer prior to LC-MS analyses. Before the analysis, the extracts were transferred to microvials in a 100 μL volume of a methanol/water mixture 1:1 (v/v).

LC-ESI/MS Analysis of Steroid Alkaloid Glycosides in Tuber Extracts. The LC-ESI/MS analyses were performed using a Waters/Micromass (Manchester, UK) ZQ mass spectrometer. The instrument was coupled with a Waters model 2690 HPLC pump (Milford, MA, USA). The samples were injected using an autosampler onto a Superspher 100 RP-18 (5 mm) column (250 × 2 mm) (Merck, Darmstadt, Germany). The flow rate was 0.2 mL min^{-1}. Steroid alkaloid glycosides were separated using a water/acetonitrile gradient. The following solvent system was applied: (A) 95% H_2O, 4.5% MeCN, 0.5% HCOOH, v/v/v; (B) 95% MeCN, 4.5% H_2O, 0.5% HCOOH, v/v/v. A linear gradient from 100% A to 100% B in (A+B) within 15 min was applied, followed by isocratic elution during 10 min with 100% B. The injection on the LC column was 3 μL from 100 μL volume in which extract samples were dissolved. The ESI source potentials were as follows: capillary, 3 kV; lens, 0.5 kV; extractor, 4 V; the cone voltage was changed during analyses from 30 to 65 V. A higher cone voltage value permitted an increase of collision-induced dissociation of protonated molecules, as well as the enhancement of protonated molecule fragmentation. The source temperature was 120 °C, and the desolvation temperature was 300 °C. Nitrogen was used as the nebulizing and desolvation gas at flow rates of 100 and 600 L h^{-1}, respectively. Glycoalkaloids were identified on the basis of their MS spectra and retention times.

Measurement of the DFR Transgenic Potato Plant Resistance to Pathogens. *Assessment of the Tuber Resistance toward Soft Rot.* *E. caratovora* subsp. *atroseptica* (Eca) bacterial strain at the concentration of 5 × 10^8/mL was used for infection of control and transgenic tubers prepared according to the method described in ref 20. The tubers were incubated for 6 days at 20 °C. After that time, the degree of infection was assessed on the basis of the rot spot diameter measured in millimeters.

Assessment of PVY0 Viral Infection. Leaves were frozen in liquid nitrogen and ground in a mortar to powder, and then 0.5 mL of buffer containing 50 mM Tric, pH 8.0, 1 mM EDTA, 12 mM 2-

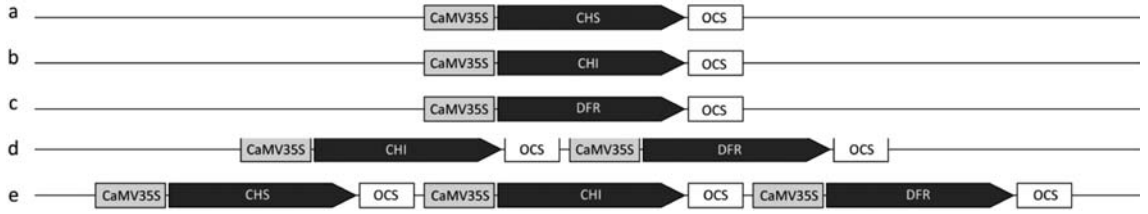

Figure 1. Schematic representation of genetic constructs used in transgenic potato plant generation: CHS, chalcone synthase (a); CHI, chalcone isomerase (b); DFR, dihydroflavonol reductase (c); W95, two-gene construct (CHI and DFR) (d); W92, three-gene construct (CHS, CHI, DFR) (e).

mercaptoethanol, and 0.05% (w/v) PMSF was added to the ground material. After centrifugation, the extracted proteins were measured in the supernatant according to the Bradford method.[21] Eighty micrograms of proteins were applied onto a 12.5% SDS polyacrylamide gel and separated electrophoretically. The proteins were then transferred onto a nitrocellulose membrane, and immunodetection of PVY0 coat protein (31 kDa) was conducted according to ref 22.

Nutritional Experiments on Laboratory Rats. The nutritional experiment was conducted at the Department of Animal Nutrition and Feed Science, Warsaw University of Life Sciences SGGW. Twenty male rats from the outbred IF_2Jaz herd with a mean body weight of 220 g were allotted to two groups (n = 10) and were kept in individual cages (enabling the control of food intake) in a room with a constant temperature of 21 °C, humidity of 60%, and a 12 h light/dark cycle.

During the 4-week-long experiment, the rats were administered an ad libitum isoprotein diet containing 30% DM of steamed and dried (50 °C) tubers of DFR-transgenic and nontransgenic Desiree variety potatoes. All diets were supplemented with 3.5% mineral mixtures (AIN-93, ICN). The body weights of the experimental animals were checked once a week, and their health status was observed every day.

On termination of the feeding experiment and after a 12 h fast, the rats were weighed and killed by ketamine overdose (50 mg kg^{-1} bw). Blood to be analyzed was sampled from the heart, whereas selected internal organs (brain, liver, small intestine, kidneys, and heart) were prepared and weighed. Liver and brain tissues were frozen and kept at a temperature of −70 °C until subjected to chemical analyses.

During the 7 days of the digestibility experiment, the total collection of feces was performed. Feces were frozen, dried at 60 °C, and, after removal of hair, ground and analyzed. The chemical composition of the diets and feces (crude protein, starch, crude fiber, and fat) was determined according to an AOAC method (1996).

Hematological, Biochemical, and Immunological Parameters of Rat Blood. Blood for morphological analyses was collected in tubes containing anticoagulant (EDTA) and assayed with standard laboratory methods using a hematology analyzer ABACUS, Diatron. For biochemical and immunological analyses, blood was centrifuged (4500 rpm , 10 min), and serum was frozen and stored at −70 °C. Biochemical parameters in blood serum were assayed using spectrometry with a Vitros analyzer, Ektachem DT-60-II system with a DT, DTE, and DTSC module with use of slide collection of Johnson & Johnson Clinical Diagnostics. Indicators of nonspecific immunity state was assayed with flow cytometry method (Becton-Dickinson) in samples of heparinized blood using ORPEGEN Pharma kits: FAGOTEST and BURSTTEST. Concentrations of IgE, IgA, IgM, and IgG in blood serum were determined using a chemiluminescent method with an Immulete 2000 analyzer. IL-4 serum concentration was analyzed with an ELISA method with R&D System according to the producer's protocol. Absorbance detection at 405 nm was performed after 30 min of incubation with an Anthos 2010 microplate reader (Biochrom Ltd.). TNF-α concentration was assayed with an ELISA method using a Peprotech kit and 96-well microplates (Bethyl Lab), according to the manufacturer's protocol. The reaction was stained with ABTS dye (Sigma). Reading was done at 405 nm using an Anthos 2010 microplate reader. The plate was incubated after coating for 24 h.

Analysis of the Oxidative and Antioxidative Status of Rat Organism. TBARS, substances reacting with thiobarbituric acid, were assayed in brain and liver tissue with a spectrophotometric method (1,2,3,3-tetraethoxypropane (TEP) was used as standard). Reading was performed at 532 nm with a UNICAM 5625 UV−vis spectrophotometer.

Nitrates and nitrites in brain and liver tissue were assayed with HPLC with a conductometric detector (Waters) according to the Accredited Analytic Center of SGGW procedure (PB 1-3, ed. 2; 25.05.2005).

The activity of superoxide dismutase (SOD) in erythrocytes (blood collected to sodium heparin, centrifuged at 3000 rpm, lysed with deionized water) was assayed using a colorimetric method, with a RANSOD kit (Randox Laboratories Ltd.). The method uses xanthine and xanthine oxidase to produce superoxide radical, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT). The produced formazine concentration results from the intensity of superoxide molecule quenching by SOD. Absorbance at 505 nm was monitored with a Coba Mira analyzer (Roche).[23] SOD activity was expressed as units per milliliter of blood.

The activity of glutathione peroxidase (GPx) in blood was assayed with a colorimetric method using a RANSEL kit (Randox Laboratories, Ltd.) according to the following method: GPx catalyzes glutathione oxidation by cumene hyperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) undergoes transformation to its reduced form (GSH) with simultaneous oxidation of NADPH to NADP. Decrease of NADPH absorbance gives a reliable indication of glutathione peroxidase activity. Absorbance was read with a Coba Mira analyzer (Roche) at 340 nm.[23] GPx activity was expressed as units per milliliter of blood (1 U = 1 μmol NADPH released during 1 min).

Total antioxidant status (TAS) of rat blood was assayed with a colorimetric method using an assaying kit from Randox Laboratories Ltd. The test uses the reaction of one-electron oxidation of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) by hydrogen peroxide. The plasma total antioxidant capacity was expressed in millimoles per liter. Reading was done with a Coba Mira analyzer (Roche) at 600 nm.[24]

Histopathological Analyses. For histopathological analyses, a section of right lateral liver lobe was sampled from laboratory rats. The preparations were fixed in 10% buffered formalin solution. The fixed preparations were sliced into 4 μm slices and stained by using the hematoxylin−eosin method and evaluated under a BX-41 Olympus microscope. Morphologic assessment of liver cells was performed, and subsequently unchanged and necrotic hepatocytes were counted. Additionally, staining with Sudan III to detect lipid presence in hepatocytes was performed.

Statistical Analysis. The data obtained were statistically analyzed using the t test for independent samples with use of STATISTICA 10 software (StatSoft, Poland). Results were statistically significant at $p < 0.05$. The animal study results were subjected to statistical analysis by the single-factor analysis of variance ANOVA and Duncan's range test, using the Statgraphic 4.1 Plus software package (Statpoint Technologies, USA).

RESULTS

Transgenic Plants. Transgenic potato plants were recently generated with overexpression of key enzymes of the flavonoid biosynthesis pathway, chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavonol reductase (DFR), CHI, and DFR (W95), and all three genes, that is, CHI, CHS, and DFR (W92)[25,26] (Figure 1).

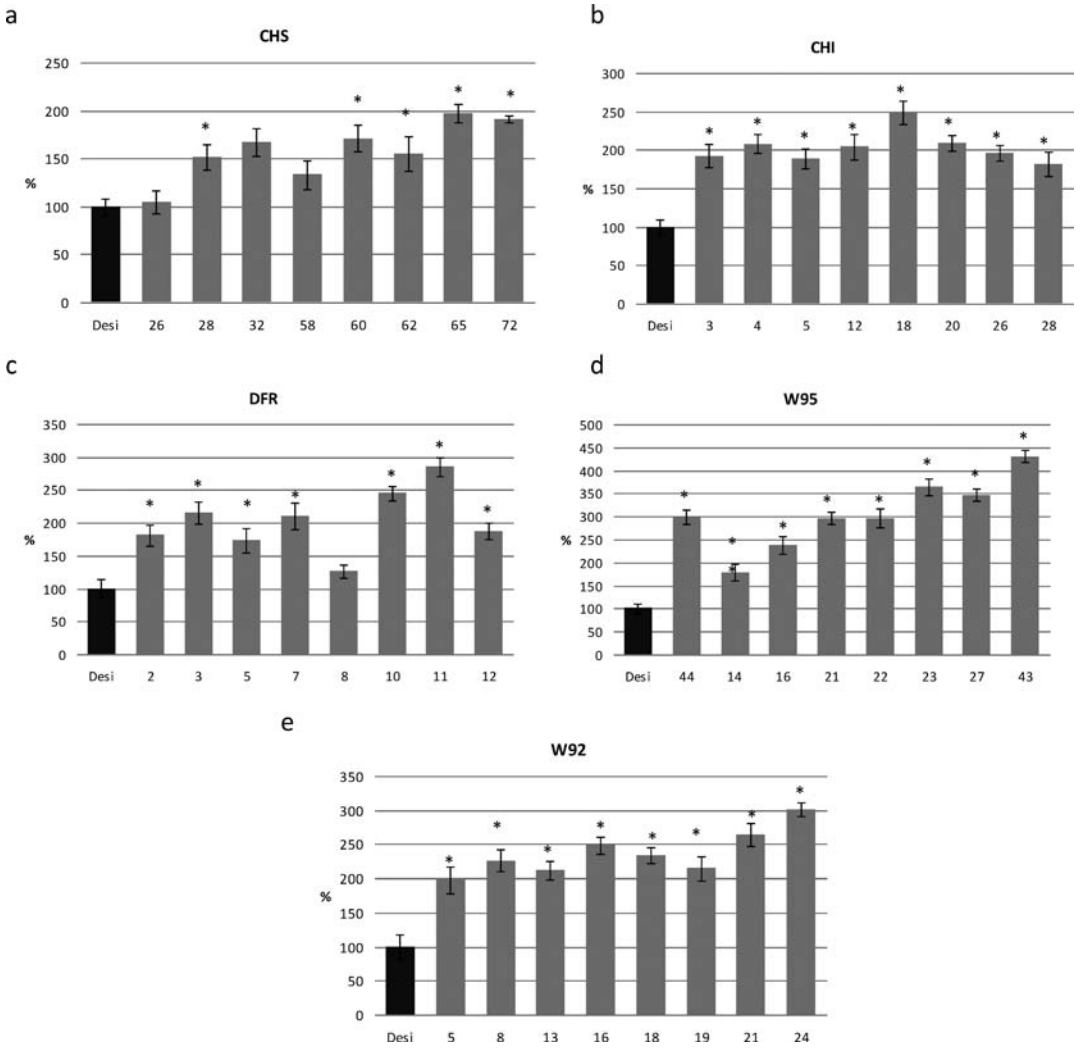

Figure 2. Densitograms of Northern blot analysis of transgenic potato plants with overexpression of chalcone synthase (CHS) (a), chalcone isomerase (CHI) (b), dihydroflavonol reductase (DFR) (c), CHI and DFR (W95) (d), and CHS, CHI, and DFR (W92) (e). Wild-type control potato plant is designated Desi. Different transgenic lines for each transgene are numbered. Results are the mean ± SD (n = 3). Asterisks (∗) indicate values that are significantly different from the wild type plants (Desi) at $p < 0.05$.

We have verified the presence of transcripts of the previously introduced genes. For that purpose we performed Northern blot analysis. All transgenic lines with gene overexpression showed an elevated level of the proper transcript. The data are presented as densitograms of bands after Northern blot analysis normalized to the 18S ribosomal subunit (Figure 2). As we have confirmed the transgenicity of the previously produced plants, we chose the line of each transgene that exhibited the strongest signal in Northern blot analysis (line 65 for CHS, line 18 for CHI, line 11 for DFR, line 43 for W95, and line 24 for W92) and submitted it to further analyses.

Analysis of Anthocyanin and Phenolic Acid Contents in CHI, CHS, DFR, W92, and W95 Transgenic Plants. Because the goal of this study was to determine the most efficient way to elevate flavonoid and phenolic acid levels, as well as antioxidative properties, the transgenic plants were analyzed and compared. Measurement of phenolic acids in tubers showed that the most intense increase in chlorogenic acid was observed for DFR transgenic line, and it was 177.7% of the wild-type control and was similarly large for CHI and W92 transgenic lines (172.3 and 171.9%, respectively). For caffeic acid, the highest increase was observed for the CHI transgenic line; however, in this case, the elevation was not as strong as in the case of chlorogenic acid and reached 135.8% of the control.

The levels of two main anthocyanins increased in all, except CHS transgenic line, in which they were similar as in the control. In CHI, DFR, W95, and W92 transgenic lines the content of petunidin was from 3.5-fold to almost 7-fold higher than in the control. Similarly, in the same lines, the level of pelargonidin derivative was from 3- to 7-fold higher in comparison to the control. The strongest elevation in petunidin derivative level was observed for W92 plants, whereas in the level of pelargonidin derivative for DFR plants. The data on phenolic acid and anthocyanin content in transgenic lines in comparison to the control are presented in Figure 3.

Analysis of Antioxidant Potential. Growing plants have to manage different unfavorable environmental conditions, both biotic and abiotic, including drought, cold, UV radiation, and, importantly, microbial infections. The plants produce a number of biochemical compounds to prevent destructive influence of the environmental stresses, with antioxidants being among them. Stress factors lead to the increase of oxygen reactive form in plant cells,[27] and their removal depends on the availability of antioxidant compounds. In potato, the main antioxidants are

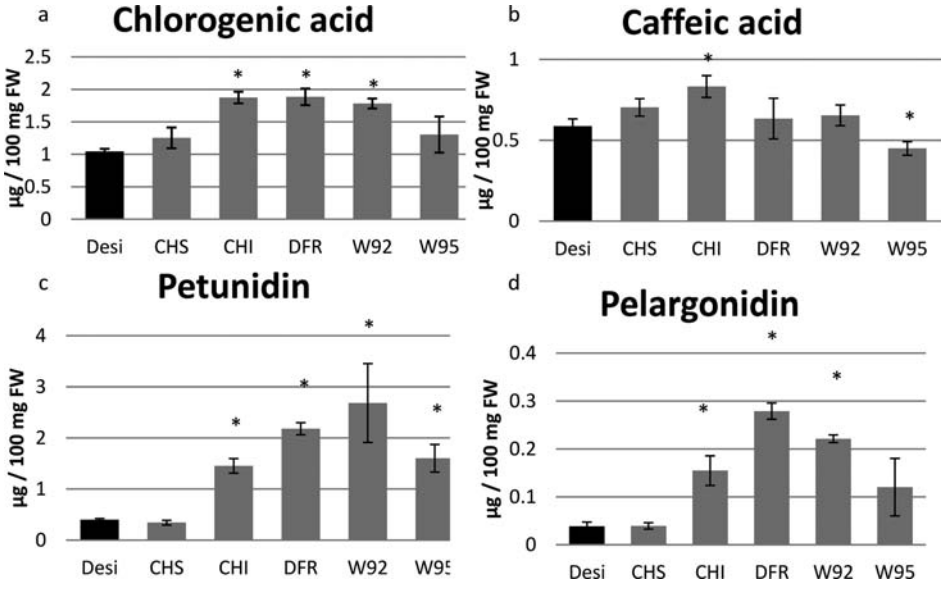

Figure 3. Levels of chlorogenic (a) and caffeic (b) acids and anthocyanin petunidin (c) and pelargonidin (d) derivatives in epidermal tuber extracts from transgenic potato plants. The phenolic compound content was measured in field-grown wild type (Desi) and transgenic potato tubers. The plants were transformed with triple (W92, containing all three cDNAs), double (W95, containing CHI and DFR cDNAs), and single (CHS, CHI, or DFR) gene constructs in sense orientation. Data represent chosen lines of each transgene. Tuber material was frozen in liquid nitrogen and examined in enzymatic assay and compared to the control (Desi). Results are the mean ± SD ($n = 6$). Asterisks (∗) indicate values that are significantly different from the wild type plants at $p < 0.05$.

vitamin C, phenolic acids, and anthocyanins. The observed increase in phenolic acids was accompanied by a 12.5% vitamin C decrease in DFR plants compared to the control. The measured antioxidant status of methanol extracts of the selected transgenic lines in comparison to the control (Figure 4) shows that the DFR

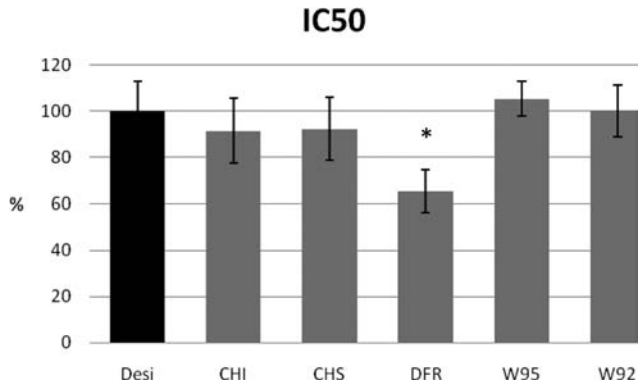

Figure 4. Antioxidant potential of control (Desi) and transgenic plant overexpressed flavonoid biosynthesis enzymes. The analysis of tuber extract was performed as specified under Materials and Methods. The plants were transformed with single (CHS, CHI, DFR), double (W95, containing CHI and DFR cDNAs), and triple (W92, containing all three cDNAs) gene constructs in sense orientation. The cDNAs encoding chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol reductase (DFR) were used for construct preparation. The mean value ($n = 3$) ± SD is presented. Asterisks (∗) indicate values that are significantly different from the wild type plants at $p < 0.05$.

overexpressing transgenic line discloses the best antioxidative potential, better by 35% than the control. Phenolic compounds, notably phenolic acids, reveal strong antioxidant properties[28] compared to such evident antioxidants as vitamin C or glutathione.[29] Although anthocyanidins (cyanidin, delphinidin, petunidin, malvidin) show lower antioxidant potential than vitamin C, their potentials are still better than that of glutathione. However, they are 3 times lower in glycosylated forms.

Productivity of DFR Transgenic Potato Plants. The DFR transgenic plants have the best antioxidant potential among all of the transgenic plants studied in this work, which corresponds to the highest level of phenolic antioxidants. Therefore, we have selected this transgene for further studies. We investigated the productivity of the potatoes, which is a total mass of tubers per plant, mean mass of a tuber, and the number of tubers per plant. The results are presented in Figure 5. The number of tubers dropped compared to the control by 40−45%. Therefore, although the mass of a single tuber did not change in the case of DFR, the total tuber mass decreased to 53% of the control.

Starch and Protein Assay in DFR Transgenic Potato Plants. As the productivity of the transgenic plants decreased, we wanted to check whether the content of starch, the most important potato product, had changed in the DFR plants. It turned out that the level of starch in DFR potato tubers decreased to 76.6% of the control (Figure 6).

The main nutritional tuber component, constituting >60% of the dry mass, is starch. Potato protein, despite its relatively low concentration in potato tuber, is an important nutritional component, one of the most valuable among plant proteins, which results from their amino acid composition. DFR transgenic tubers exhibit an elevated level of protein by 70% in comparison to the control (Figure 6).

DFR Transgenic Potato Plant Resistance to Pathogens. Among bacterial pathogens of potato plant, Gram-negative *E. carotovora* represents one of the most dangerous threats for potato tubers, causing bacterial soft rot. The bacteria enter a tuber through natural pores or wounds on the surface and secrete various extracellular enzymes, such as peptidases, cellulases, and proteases, which cause cell wall degradation, leading to tissue maceration.[30] Control and DFR transgenic potato tubers were treated with *E. carotovora*, and the size of rot spot was observed. Results are presented in Figure 7. After the same amount of

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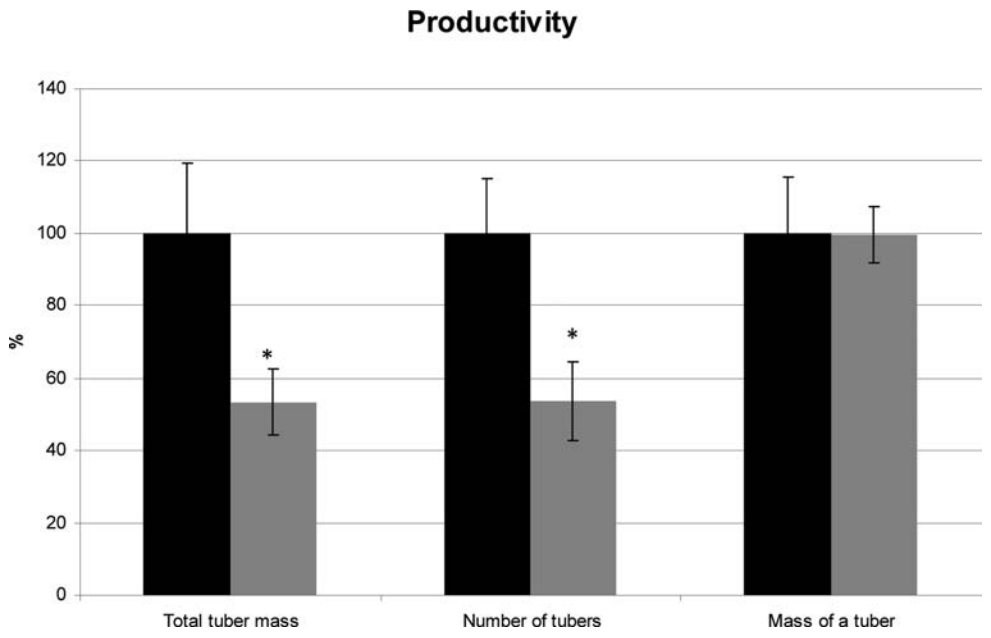

Figure 5. Analysis of productivity of the control (Desi) and transgenic (DFR) potatoes from greenhouse-grown plant. The results are the mean value of 10 independent transgenic plants for each transgenic line and 10 independent control plants. Bars represent standard deviation (n = 10). Asterisks (∗) indicate statistically significant data (p < 0.05).

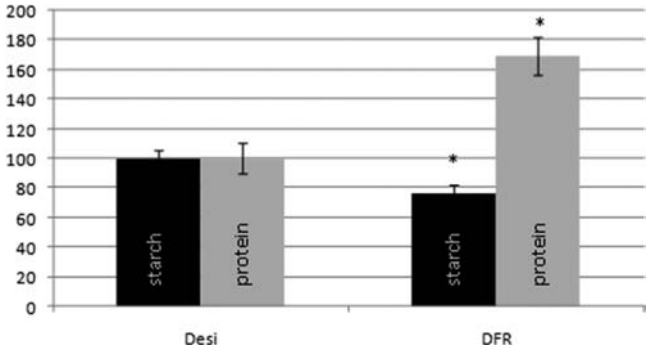

Figure 6. Levels of starch (black columns) and proteins (gray columns) in the control (Desi) and transgenic (DFR) potato tubers. Bars represent standard deviation (n = 3). Asterisks (∗) indicate statistically significant data (p < 0.05).

inoculation time of tubers with the bacteria, the disease development was earliest in Desi, control plants. In the case of the DFR transgenic plant, the resistance was improved in comparison to the control, and the size of the rot spot was by 41% smaller than in the wild type.

Serious damage in potato cultivation is caused also by potato virus Y (PVY0). It causes potato leaves to curl and fall off, necrotic potato mosaic, and, in the case of tubers, formation of irregular, small, dark necrotic spots.[31] Analysis of phenotypic symptoms is often difficult and equivocal and depends on the conditions during culturing of the infected potatoes. Therefore, to confirm the presence of PVY0 in the plant, a specific immunodetection method was used. It was based on the recognition of virus coat protein (CP) isolated from infected and noninfected leaves collected 7, 14, and 21 days after inoculation (Figure 7) and Western blot technique. The CP protein was present in the first week of the experiment in all infected leaves. After 2 weeks, it appeared in the noninfected leaves of the control due to the migration of the virus within plant tissues. After 3 weeks, the CP protein was present in the noninfected leaves of

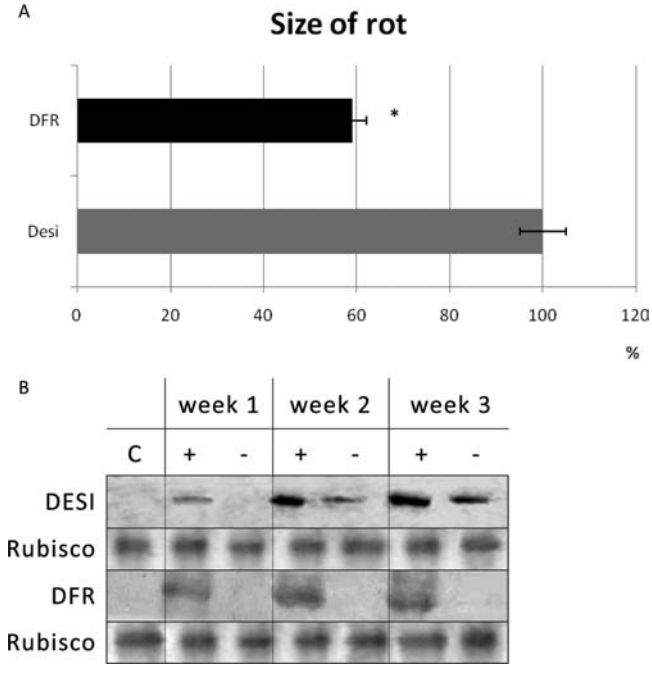

Figure 7. (A) Size of the rot spot developed after infection of control (Desi) and transgenic (DFR) tubers with *Erwinia carotovora*. Bars represent standard deviation (n = 3). Asterisks (∗) indicate statistically significant data (p < 0.05). (B) Immunodetection of PVY0 coat protein isolated from control (Desi) and transgenic (DFR) plants infected with the virus. Leaves were collected 1, 2, and 3 weeks after inoculation. Each analysis used protein extracts isolated from infected (+) and noninfected (−) leaves. "C" represents the result for a proper plant (respectively, control or transgenic), which was treated with pure water in place of the virus. The amount of 80 μg of proteins was applied into each well. Rubisco is shown as a control of protein loadings.

the control and DFR transgenic plants, although the signal in the DFR leaves was very weak. The experiment showed that plants characterized by elevated phenolic acid and anthocyanin

synthesis have a more efficient mechanism of resistance to the pathogen. Multiplication and spread of the virus in this plant organism was considerably hampered.

Analysis of Alkaloid Content. Many plants of the *Solanaceae* family, including the potato, contain natural toxicant steroidal glycoalkaloids, such as solanine and chaconine. Glycoalkaloids may play different, unrecognized biological roles in the plant cell, their content regulated by several biotic and abiotic stimuli including environmental factors such as light, humidity, temperature, and mechanical injury or wounding by insects.[32] These compounds are composed of identical aglycones, but differ in sugar moieties. The poisoning character of glycoalkaloids results from their anticholinergic activity in the central nervous system and their ability to induce cell membrane breakdown by activation of cell digestion systems.[33] The toxic character is of special importance for potato for the common use of tubers as primary food source for humans and animals. The level of glycoalkaloid extracted from tuber was measured in control (Desi) and DFR, transgenic plants. Chaconine content was by 71.5% higher in the selected DFR transgenic line than in the control nontransformed plants (Desi). The solanine level increased in DFR by 75.2% in comparison to the control (Table 1).

Table 1. Steroid Glycoalkaloid Contents (α-Chaconine and α-Solanine) Analyzed in the Transgenic DFR Tubers and Control (Desi)[a]

alkaloid	Desi	DFR
α-chaconine (mg/100 g DW)	124.4 (11.2)	213.4 (14.6)*
α-solanine (mg/100 g DW)	29.4 (9.9)	51.6 (5.2)*

[a]Data are the mean of three samples (standard deviations are presented in parentheses). Asterisks mark statistically significant data ($p < 0.05$).

Nutritional Studies on Laboratory Rats. *Influence of Fodder Supplemented with Dried DFR Potato Tubers on Rat Digestibility and Body Mass Gain.* It is considered that transgenesis, independent from the intended goal, may lead to unexpected changes in tuber composition, which, because of the scale of utilization of the potato plant, constitutes a potential threat for the health of humans and animals. Moreover, elevated levels of steroidal glycoalkaloids (solanine, chaconine) in DFR transgenic plants may present a serious danger in the diet.[34]

Apparent digestibility of nutritional components containing 30% of transgenic potato tuber dry mass was assessed in laboratory rats. No statistically significant differences in values of apparent digestibility indices of crude protein, starch, crude fiber, and fat were stated (data not shown). Rats fed for 4 weeks with control and transgenic potato tubers did not exhibit statistically significant differences in body weight gain, neither per day nor in total (Table 2).

Hematological Parameters of Rat Blood. Study of the blood parameters of rats fed fodder supplemented with 30% of dried DFR tubers showed that the level of red blood cells (RBC) was unchanged in comparison to the wild type control. However, a statistically significant decrease (by 30.2%) in the content of leucocytes (WBC) was observed. Lymphocytes constituted 95.25% of total white blood cell in the blood of rats fed wild type tubers, and their concentration was lower (78.25%) in the DFR group. The change was not statistically significant. The contents of neutrophils and eosinocytes were 3.6 and 0.13%, respectively, in wild type tuber fed rat blood and were elevated in the case of transgenic tubers, 21.8 and 0.83%, respectively. The difference in neutrophil content between both groups of rats was statistically significant ($p < 0.05$) (Table 3).

Table 2. Rat Body Weight Gain Indices after Feeding 30% Transgenic (DFR) and Nontransgenic (Desi) Potato Dried Tuber[a]

parameter	Desi	DFR
initial body wt (g)	221.48 (8.91)	226.23 (7.97)
final body wt (g)	308.23 (3.51)	302.20 (2.12)*
daily body wt gain (g)	3.06 (0.11)	2.81 (0.09)*

[a]Data are the mean of 10 samples (standard deviations are presented in parentheses). Asterisks mark statistically significant data ($p < 0.05$).

Table 3. Selected Hematological Parameters Measured in Blood of Rats after Feeding 30% Transgenic (DFR) and Nontransgenic (Desi) Potato Dried Tuber[a]

parameter	Desi	DFR
RBC ($10^{12} \times L^{-1}$)	9.17 (0.32)	8.86 (0.28)
WBC ($10^{9} \times L^{-1}$)	8.12 (1.1)	5.67 (1.2)*
lymphocytes (%)	95.25 (20.91)	78.25 (13.63)
neutrophils (%)	3.60 (0.98)	21.80 (3.25)*
basocytes (%)	0.17 (0.05)	0.14 (0.09)
eosinocytes (%)	0.13 (0.27)	0.83 (0.45)
phagocyting monocytes (%)	39.02 (6.85)	19.01 (3.49)*

[a]Data are the mean of 10 samples (standard deviations are presented in parentheses). Results marked with an asterisk (*) are statistically significant at $p < 0.05$.

Biochemical Parameters of Rat Blood. Diet with 30% addition of dried transgenic tubers influenced the metabolism of studied rats, which was reflected in biochemical blood parameters. Statistically significant increases were observed in the case of asparagine transferase and alanine transferase activities (AspAT and ALAT, respectively), by 47 and 41.3%, respectively. Similarly, the activity of lactate dehydrogenase activity (LDH) increased significantly by almost 32% relative to the control. Variations in the blood parameters connected with lipid metabolism were determined. Total cholesterol in blood decreased significantly by 19.6% in rats fed DFR dried tuber addition in comparison to the control. Similarly, triglyceride concentration dropped by 51.4%. Other biochemical blood parameters (total protein (TP), albumin (ALB), globulin (GLOB), blood urea nitrogen (BUN), creatinine concentration (CREA), glucose (GLC)) did not change significantly (Table 4).

Immunological Parameters of Rat Blood. Differences in concentration of IgE, which are connected with allergic reactions, assayed in blood of rats fed addition of transgenic dried tubers were not observed in rats fed with both wild type and transgenic dried tubers. Similarly, no statistically significant changes in the remaining classes of immunoglobulins were determined. Concentrations of IL-4 and TNF-α did not exceed the threshold of ELISA tests (5 pg mL^{-1}) in any case.

Neutrophil oxidative activity (BURSTTEST) in the blood of rats receiving diet with 30% content of transgenic dried tubers was significantly higher regardless of the used chemotactic factor than in the case of wild type control (Table 5).

Percent of phagocytizing monocytes in the blood of rats of the DFR group was significantly lower (by 51.3%) compared to the control (Table 3).

Table 4. Chosen Blood Serum Biochemical Indices Measured in Rats after Feeding 30% Transgenic (DFR) and Nontransgenic (Desi) Potato Dried Tuber[a]

parameter	Desi	DFR
TP ($g \times L^{-1}$)	62.01 (0.94)	61.2 (0.33)
ALB ($g \times L^{-1}$)	37.82 (0.70)	39.00 (1.14)
GLOB ($g \times L^{-1}$)	24.20 (1.21)	22.21 (1.11)
BNU ($mmol \times L^{-1}$)	5.44 (0.28)	6.28 (0.77)
AspAT ($U \times L^{-1}$)	262.4 (10.21)	385.6 (19.68)*
ALAT ($U \times L^{-1}$]	31.00 (3.27)	43.80 (9.57)*
CREA ($\mu mol \times L^{-1}$)	34.47 (1.44)	32.71 (1.79)
LDH ($U \times L^{-1}$)	895.7 (51.44)	1142.5 (62.47)*
GLC ($mmol \times L^{-1}$)	6.41 (0.17)	6.66 (0.31)
CHOL ($mmol \times L^{-1}$)	1.53 (0.13)	1.23 (0.10)*
TRIG ($mmol \times L^{-1}$)	1.05 (0.22)	0.51 (0.19)*

[a]TP, total protein; ALB, albumin level; GLO, globulin; BUN, blood urea nitrogen; AspAT, asparagine transferase; ALAT, alanine transferase; CREA, creatinine; LDH, lactate dehydrogenase; GLC, glucose; CHOL, cholesterol; TRIG, triglycerides. Data are the mean of 10 samples (standard deviations are presented in parentheses). Results marked with an asterisk (*) are statistically significant at $p < 0.05$.

Table 5. Neutrophil Oxidative Activity (BURSTTEST) in Blood of Rats Receiving Diet with 30% Content of Transgenic (DFR) and Nontransgenic (Desi) Dried Tubers[a]

	% of oxidizing cells	
chemotactic factor	Desi	DFR
E. coli	0.10 (0.08)	0.54 (0.12)*
fMPL	0.05 (0.00)	0.24 (0.12)*
PMA	1.61 (0.57)	4.68 (0.97)*

[a]*E. coli*, *N*-formylo-methionylo-leucylo-phenyloalanine (fMPL), and phorbol 12-myristate 13-acetate (PMA) were used as chemotactic factors. Data are the mean of 10 samples (standard deviations are presented in parentheses). Statistically significant differences ($p < 0.05$) are marked with an asterisk (*).

Oxidative and Antioxidative Status of Rat Organism. Genetic modification of plants administered in the diet did not have an effect on antioxidative enzyme activity, for either superoxide dismutase or glutathione peroxidase (data not shown). Furthermore, total antioxidant status (TAS) in the blood of rats from the DFR group was lower, compared to the control group, by 10%. The content of nitrates and nitrites, which are the products of protein degradation, dropped in the DFR group by 20% compared to the control. The concentration of the compounds interacting with TBARS, which results from the lipid degradation state, did not change regardless of the administered diet, in either the brain or liver tissue (Table 6).

Micro- and Macroscopic Tissue and Organ Assessment. The presence of DFR dry tuber addition in diet did not influence the weight of examined organs (brain, liver, kidneys, intestines, heart) (Table 7). Similarly, macroscopic evaluation of organs directly after preparation did not reveal changes caused by the diet in comparison to the rats fed with wild type dried tuber addition.

Histopathological assessment of liver originating from rats of the DFR group showed a statistically significant higher number of normal hepatocytes in comparison to the control group. Additionally, the microscopic picture of hepatocytes of rat liver revealed a statistically significant lower number of necrotic cells in the DFR group compared to the wild type control. No lipids were observed in hepatocytes.

Table 6. Nitrate and Nitrite Contents and Thiobarbituric Acid Reacting Compounds (TBARS) Assayed in the Brain and Liver of Rats Fed 30% Supplementation of Transgenic (DFR) and Nontransgenic (Desi) Potato Dried Tubers[a]

parameter	Desi	DFR
TAS ($mmol\ L^{-1}$)	0.990 (0.051)	0.899 (0.031)*
NO_2^-, NO_3^- ($mg\ kg^{-1}$) in brain	1.67 (0.21)	1.30 (0.39)
NO_2^-, NO_3^- ($mg\ kg^{-1}$) in liver	1.59 (0.09)	1.32 (0.15)
TBARS ($mmol\ g^{-1}$) in liver	0.285 (0.007)	0.283 (0.009)
TBARS ($mmol\ g^{-1}$) in brain	0.282 (0.004)	0.279 (0.005)

[a]Total antioxidant status (TAS) was measured in the blood of rats of both studied groups. Data are the mean of 10 samples (standard deviations are presented in parentheses). Statistically significant differences ($p < 0.05$) are marked with an asterisk (*).

Table 7. Relative Mass (Grams per 100 g) of Selected Organs of Rats Fed 30% Supplementation of Transgenic (DFR) and Nontransgenic (Desi) Potato Dried Tubers[a]

organ	Desi	DFR
brain	0.584 (0.027)	0.561 (0.047)
liver	2.813 (0.087)	2.912 (0.053)
kidneys	0.651 (0.007)	0.662 (0.008)
heart	0.351 (0.092)	0.502 (0.048)
small intestine (empty)	1.250 (0.014)	1.270 (0.009)

[a]Data are the mean of 10 samples (standard deviations are presented in parentheses). Asterisks mark statistically significant data ($p < 0.05$).

DISCUSSION

The potato plant, as one of the most frequently cultivated crops in human agriculture, constitutes a great source of nutritional value, with starch being the most important component. Phenolic compounds are strong antioxidants and, together with ascorbic acid, tocopherols, and carotenoids, protect the human organism against oxidative stress.[35] Flavonoids and phenolic acids are the compounds that play a significant role not only in the human diet but also in plant growth and development. In both cases, they constitute a crucial element of antioxidative apparatus protecting against reactive oxygen forms.[36] On the basis of analyses of the influence of flavonoid biosynthesis enzyme gene overexpression (i.e., chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavanol reductase (DFR) or double combination (CHI/DFR), or using all three enzymes in one genetic construct) on the level of those compounds and the antioxidative properties of potato plants, we demonstrated that the enzyme most suitable for manipulation of the phenolic compound level in potatoes is DFR. However, despite a strong increase in phenolic compound content, we have observed only small changes in the antioxidative capacity, especially in CHS, CHI, W92, and W95 type plants. The molecular background for the lack of clear correlation between phenolic content and antioxidant capacity is as yet unknown in detail, but certainly glycosylation level of these metabolites might play a primary role. We believe that the excess of phenolics produced in the transgenic plants for the most part undergoes glycosylation. Using a constitutive CaMV35S promoter in DFR overexpression allowed anthocyanidin content increase (to 5−7-fold of the control). Moreover, the DFR plants exhibited higher resistance to bacterial and viral infections. However, we have observed negative effects in the plant, such as growth reduction, decrease in the number of tubers, and starch level drop. We suspect that these deleterious effects may result from the excess

of phenolic compound aglycones. Under normal conditions, the majority of flavonoids occurs in plants in their less active form of glycosides. It is postulated that under stress conditions, for example, fungal or bacterial infection, the pool of stable, nonreactive flavonoid glycosides is activated by cutting off the sugar moiety by specific glycosidases.[37] As a consequence, the concentration of highly reactive aglycones, capable of neutralizing free radicals, increases.

One of the ways to solve this problem could be overexpression of glycosyltransferase in plants with an already elevated phenolic compound level. Maintaining the phenolics in their glycoside form would diminish their toxic side effect in plants and increase their stability. It was previously demonstrated that overexpression of glycosyltransferase in potatoes led to the elevation of anthocyanins by 41%, increased resistance to pathogens, and raised productivity.[38] On the basis of those results, it was anticipated that GT overexpression in DFR plants should give an elevated level of flavonoid glycoside derivatives. Indeed, such plants were generated.[26] The glycosylation process increased the stability of flavonoids and contributed to the decrease of their reactivity and toxicity. The productivity of DFR plants was restored to the initial level of the control plants. Another approach toward the undesirable effect of phenolic compounds would consider placing the transgene (DFR) under a tissue-specific promoter and therefore directing the assumed overproduction to a specific organ. Transgenic plants with overexpression of DFR under B33-tuber specific promoter were recently produced.[38] The plants showed elevated starch content and increased productivity.

Determination of the influence of the increased level of phenolic compounds in the diet containing transgenic potato plants with overexpression of DFR is of high importance for the nourishment and health values of these potatoes. Incorrect organism response to the components of diet may be a toxic reaction, allergy, or food intolerance, which are accompanied by symptoms such as pain, vomiting, and diarrhea. The organism reacts with changes in the immune system, such as the number and activity of immune cells. In our study rats fed 30% DFR potato tuber diet showed mainly some effects of allergic reaction to dietary components. Administration of nutrient allergen in the diet may cause extensive activation of mast cells, eosinocytes, basocytes, and T lymphocytes, which stimulate the organism to produce specific IgE antibodies. The level of innate immune system cells, neutrophils, and eosinocytes increased in the DFR group rat blood, although the IgE content remained low. In the case of lack of allergic reaction, IgE concentration is extremely low in blood serum. A high level of IgE, accompanied with normal concentrations of the remaining classes of antibodies, could be evidence of an allergic reaction.[39] A consequence of the contact of a rat organism with dietary allergen could be an increase in eosinocyte count in the blood, but the observed increase detected in rats consuming the DFR potato was statistically insignificant. Although flavonoids were shown to influence the level of cytokines, the concentration of phenolic compounds in the rat organism after administration of DFR transgenic potatoes was too low to induce expression of genes and production of proteins connected with immune reaction (data not shown). No changes in cytokine contents were observed. On the other hand, Bub *et al.*[40] observed that even a large dose of anthocyanins, which stimulates the immune system, does not influence IL-4 secretion by T lymphocytes. The mechanisms of the innate immune system are based on macrophage, neutrophil, and monocyte activity, which destroy antigens with oxygen (oxidative burst) and phagocytosis. Activation of oxygen processes is connected with generation of reactive oxygen species (ROS). Phagocytes release ROS to phagosomes and out of the cell.[41] Activation of phagocyting cells leads to ROS release, which may influence tissue or DNA degradation.[42] Both the antioxidant content (for which synthesis was modified in DFR plants) and the presence of toxic glycoalkaloids may influence the redox balance in consumer organism. Disturbance in the equilibrium between oxidation and reduction process intensity in cells is one of the parameters indicating potential toxicity of a diet.[43] The activity of ROS in cells is inhibited by antioxidants, and unbalance in redox processes, caused by increased ROS generation in response to toxic substance in diet, may lead to oxidative stress. After administration of a diet supplemented with potato dried tubers, we searched for changes in the activity of antioxidative enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GPx). The activities of the antioxidative enzymes can be stimulated by the presence of free radicals resulting from receiving toxic substances in diet.[44] We did not detect changes in the activities of the antioxidative enzymes, but we observed a significant decrease in the TAS measured in rats fed transgenic DFR potato compared to the control. This can result from the presence of toxic products (e.g. glycoalkaloids) and may be evidence of a disturbance in redox balance, caused by activation of cells involved in phagocytosis intensely releasing oxygen radicals in response to diet components.

The presence of toxic substances did not cause visible changes in the rat organism. The concentration of glycoalkaloids was not high enough to cause adverse effects. Mean liver masses of rats from both the DFR and control groups did not differ significantly, which means that changes in tuber composition caused by DFR overexpression did not lead to overgrowth of this organ, responsible for toxic substance metabolism, although such an effect was observed by Friedman *et al.*[45] after administration of alkaloids to mice. The increased level of normal hepatocytes in rats fed DFR potatoes may result from a positive influence of diet components, including flavonoids, which reduce the negative activity of glycoalkaloids. However, the glycoalkaloids could cause an increase in aspartate transaminase, alanine transaminase, and lactate dehydrogenase activities, which could be an indication of liver damage. Simultaneously, probably due to the cytoprotective effect of flavonoids, a decrease in necrosis of hepatocytes was observed.

The participation of transgenic potatoes in rat diet influenced the lipid profile in blood. Serum of DFR group rats contained decreased levels of total cholesterol and triglycerides, compared to the control, which is evidence of the beneficial influence on the blood lipid profile of a diet supplemented with DFR plants. This positive influence may result from the presence of antioxidative substances, especially anthocyanins, which protect lipid fractions from peroxidation thanks to the ability to chelate pro-oxidative metal ions and to form complexes with metals and ascorbic acid.[46,47] Our observation is confirmed in other authors' studies demonstrating the positive impact of flavonoids on lipid profile, HDL concentration increase,[48] LDL oxidation decrease,[49] and triglyceride level decrease.[50] Additionally, reduction of serum triglyceride levels caused by foods rich in polyphenol has been associated with diminished intestinal absorption of these lipids due to inhibition of the activity of pancreatic lipase[51] or to a lower microsomal transfer protein activity and apoB secretion or increase in hepatic LDL receptor expression and lipoprotein lipase activity.[52] This could modify the VLDL levels, which could

lead to reduced LDL-cholesterol content. In addition, the effect of dietary fiber cannot be omitted. It is well-known that viscous soluble polysaccharides lower total and LDL-cholesterol, attenuating or preventing hypertriglyceridemia.[53]

No influence of transgenic potato supplemented diet on parameters connected with protein metabolism was observed. No changes in blood protein profile and blood urea nitrogen concentration allow us to state the lack of a toxic effect of components contained in DFR transgenic potato supplemented diet.

In conclusion, despite a relatively high level of antinutritional or even toxic compounds (steroidal glycoalkaloids) contained in diet supplemented with transgenic potato tubers, no strong symptoms connected with deterioration of health were observed in rats The only negative change noted was elevation of liver enzyme activities, which was, however, not accompanied by liver damage. This could be an early indication of liver damage, suggesting that further studies are needed. The toxic effect of glycoalkaloids was probably modified by the significant content in the diet of antioxidative and detoxifying substances (flavonoids), which play a protective role for cells and organs. This is an indication that potatoes with increased flavonoid content are valuable food sources; however, new lines without glycoalkaloids should be developed. Transgenic plants with elevated levels of flavonoids exhibit better resistance to pathogens and, after introduction of glycosyltransferase, are not characterized by worsened productivity. As microbial diseases generate serious problems in potato production, the features exhibited by DFR::GT transgenic plants, together with their beneficial nutritional effect on the consumer, make them ideal for use in the food industry.

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ABBREVIATIONS USED

DFR, dihydroflavonol reductase; CHS, chalcone synthase; CHI, chalcone isomerase; GT, glucosyltransferase; TAS, total antioxidant status; SOD, superoxide dismutase; PGx, glutathione peroxidase; ROS, reactive oxygen species

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