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Nutritional properties of tubers of conventionally bred and transgenic lines of potato resistant to necrotic strain of *Potato virus Y* (PVY^N)⋆

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The potential effect of genetic modification on nutritional properties of potatoes transformed to improve resistance to a necrotic strain of *Potato virus Y* was determined in a rat experiment. Autoclaved tubers from four transgenic lines were included to a diet in the amount of 40% and compared with the conventional cv. Irga. The experiment lasted 3 weeks and special attention was paid to nutritional properties of diets, caecal metabolism and serum indices. Genetic modification of potato had no negative effect on the chemical composition and nutritional properties of tubers, ecosystem of the caecum, activity of serum enzymes and non-specific defence mechanism of the rats. Obtained results indicate that transgenic potato with improved resistance to PVY^N: line R1F (truncated gene coding for PVY^N polymerase in sense orientation), R2P (truncated gene coding for PVY^N polymerase in antisense orientation), and NTR1.16 (non-translated regions of PVY^N genome in sense orientation) are substantial and nutritional equivalence to the non-transgenic cultivar. Tubers of transgenic line NTR2.27 (non-translated regions of PVY^N genome in antisense orientation) increased the bulk of caecal digesta and the production of SCFA as compared to tubers of the conventional cultivar and the other transgenic clones. Taking into account some deviations, it seems reasonable to undertake a long-term feeding study to confirm the nutritional properties of tubers of transgenic lines.

Keywords: potato, genetic modification, nutritional value, serum, ceacal fermentation, rat

The potato crop may be affected by many biotic and abiotic factors, pathogens and environmental stress. When pathogens are not properly controlled, the potato yield of infected plants may be reduced by 80%. This is a serious economic problem in countries where potatoes are cultivated on large areas. In Poland, the most important potato pathogens include *Potato virus Y* (PVY), and particularly a new isolate of PVY from the subgroup of necrotic strains (PVY^N). Necrotic strains of potato virus are spreading now in Poland and have been responsible for 80–90% of PVY infection in potato fields in the last decade (Chrzanowska & Doroszewska, 1997). Potato breeding involves the application of a number of natural resistance genes to PVY originating from different *Solanum* species. Obtaining new cultivars resistant to viral diseases through classic breeding involves a transfer of desired features from wild potato species, which is a long-lasting process. Genetic transformation might be a faster and more effective way of potato breeding, improving the efficiency of pathogen control (Jondedijk *et al*., 1992).

In the last decade, the method of genetic transformation has repeatedly been used in order to improve potato characteristics, including beneficial changes in the metabolism of protein, starch and sucrose (Prescha *et al.,* 2002; Zuk *et al.,* 2003; 2005), an increase of plant resistance to pathogenic fungi (Gazendam *et al.,* 2004), bacterial pathogens (Pandey *et al.,* 2005), and most of all resistance to *Potato virus Y* (Chachulska *et al.,* 1997; Flis & Zimnoch-Guzowska, 2000; Missiou *et al.,* 2004). Information concerning the *in vivo* experiments with the genetic engineering plant products as dietary components is insufficient. In an experiment of Kosieradzka and coworkers (2004), genetic modification aimed at increasing the content of 14-3-3 protein in

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Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CK, creatine kinase; GMO, genetically modified organism; LDH, lactate dehydratase; PVY, *Potato virus Y*; PVY^N, necrotic strain of *Potato virus Y*; SCFA, short chain fatty acid; wt, wild type.

potato plants has led, on the one hand, to changes in the nutritional value of tubers, and on the other hand, the administration of 20% dried tubers to diets for rats did not negatively affect the assayed growth parameters, digestibility of nutrients, blood morphology or most blood biochemical parameters. Transgenic potatoes with soybean glycinin included in diet neither influenced the diet intake and body mass gain, nor changed blood parameters as well as masses of internal organs, compared to animals fed diets containing conventional tubers (Hashimoto *et al*., 1999a; 1999b). In another experiment of Sanhoty and coworkers (2004), there were no statistical differences in the diet intake and body mass gain between rats fed genetically modified potatoes spunta with *CryV* gene and unmodified tubers. In an experiment of Böhme and coworkers (2005), genetic transformation of inulin synthesis in potatoes resulted in depression of starch content and fibre digestibility for pigs. After a feeding period of 42 days no plant DNA or DNA specific for the genome alteration in the transgenic potato were detected in gastrointestinal digesta or in the internal organs of pigs (Broll *et al.*, 2005).

Most consumers are anxious of a potential GMO-related risk to their own or animal health. For this reason, the biological assessment of food and feeds from genetically modified plants is considered as advisable (Beever & Kemp, 2000; Zduńczyk, 2001; Aumaitre *et al.*, 2002, Flachowsky & Böhme, 2005). The present report summarizes the results from experimental feeding of rats with a diet containing a high percentage of potato tubers genetically transformed to improve resistance to a necrotic strain of *Potato virus Y*. Special attention was paid to the nutritional properties of the diet, biochemical parameters of blood including indices of non-specific defence, as well as large-intestine metabolism parameters.

MATERIAL AND METHODS

The transgenic lines of potato were prepared at the Institute of Biochemistry and Biophysics of the PAS (Warszawa, Poland) (Chachulska *et al*., 1997). Plants of cultivar Irga were transformed with viral genome sequences in order to improve their resistance to a necrotic strain of *Potato virus Y* (PVY^N). Four clones were chosen for the nutritional study:

- **R1F** and **R2P** — transgenic lines with a truncated gene coding PVY^N polymerase in the sense and antisense orientation, respectively;
- **NTR1.16** and **NTR2.27** — transgenic lines with non-translated regions of PVY^N genome in the sense and antisense orientation, respectively.

Potato tubers obtained from the genetically-modified potato lines were compared with two types of non-transgenic tubers: obtained from conventionally-bred cultivar Irga and from plants of somaclone Irga, regenerated and maintained *in vitro* under the same conditions as the transgenic lines (Irga wt). The transgenic lines and their conventional counterparts were multiplied at the Plant Breeding and Acclimatisation Institute (Research Centre Mlochow, Poland). All tubers used in the presented investigations were autoclaved (121°C, 1013 hPa, 15 min), dried (40°C) and ground. Our investigations, involving 3-week feeding experiment on rats, included the following determinations: the chemical composition of tubers and nutritional properties of diets with high (40%) content of tubers (Zduńczyk *et al*., 2005a), caecal metabolism, serum enzymes and indices of non-specific defence of rats (Zduńczyk *et al*., 2005b), and short-chain fatty acids (SCFA) production in the caecum of rats (Juśkiewicz *et al*., 2004).

RESULTS

Results in Table 1 show that the crude protein, starch and fibre content in dry matter of potato was comparable in tubers obtained from the conventional cultivar Irga, somaclone Irga and the transgenic lines. Also the content of essential amino acids in crude protein of transgenic potato was similar or higher than in tubers of the conventional cultivar Irga. A higher content of essential amino acids (i.e. leucine and lysine) was noticed for the transgenic line R2P.

Results in Table 2 indicate that supplementation diets with autoclaved potato tubers of conventional and transgenic lines did not influence the growth of animals. Feed intake as well as feed utilization were similar in all experimental groups. The activity of the enzymes analysed in the blood serum of rats was diversified not only between groups but also within each experimental group. Comparable activities of aspartate aminotransferase (AST), creatine kinase (CK) and alkaline phosphatase (AP) in all groups were measured. The activity of alanine aminotransferase (ALT) and lactate dehydratase (LDH) in the group fed with transgenic potato was within the range determined for the Irga group and the somaclone Irga group (Irga wt). Similar tendencies in the indices of non-specific resistance of rats were observed. In the groups fed diets containing tubers of the transgenic lines, the indices analysed were within the limits reported for the groups fed tubers of the conventionally bred cultivar or Irga somaclone. However, a lower percentage of phagocytic cells was found in the groups fed a diet with tubers of the transgenic lines R1F, NTR1.16 and NTR2.27.

Results in Table 3 show that in the group fed a diet with tubers of cultivar Irga and tubers of the transgenic lines a similar mass of the caecum wall

Table 1. Indices of chemical composition of autoclaved and dried potato tubers (Zdańczyk *et al.*, 2005a)

	Irga	Irga wt	Transgenic line			
			R1F	R2P	NTR1.16	NTR2.27
Dry matter, %	90.05	89.83	89.94	89.83	89.83	89.97
Crude protein, %	10.00	9.81	9.94	9.95	10.25	10.10
Dietary fibre, %	8.87	9.26	8.77	8.88	9.78	9.32
Starch, %	76.65	75.96	76.18	76.58	74.68	76.24
Phe + Tyr	6.68	6.68	6.84	7.44	6.86	6.71
Iso	3.60	3.83	3.74	3.99	3.79	3.70
Leu	5.77	5.97	5.94	6.30	6.05	5.82
Lys	4.42	4.49	4.45	4.67	4.48	4.46
Met + Cys	3.33	3.37	3.42	3.36	3.43	3.46
Thr	3.50	3.59	3.60	3.66	3.56	3.44
Val	5.53	5.59	5.49	5.85	5.70	5.49
ΣEAA[1]	38.14	38.90	38.87	40.88	39.52	38.45
ΣEAA, % ΣAA[2]	44.1	43.8	44.6	45.1	44.2	44.5

[1]ΣEAA, total essential amino acids; [2]ΣAA, total amino acids.

Table 2. Results of feeding experiment[1], activity of serum enzymes[2] and indices of non-specific resistance of rats[3] (Zdańczyk *et al.*, 2005b)

	Irga	Irga wt	Transgenic clone				S.E.M.
			R1F	R2P	NTR1.16	NTR2.27	
IBW, g	122.7	122.7	122.6	122.6	122.6	122.7	0.01
FBW, g	213.4	212.6	213.7	212.1	209.7	212.1	1.21
DI, g/day	17.70	17.45	17.21	17.57	17.32	17.45	0.06
BWG g/day	4.32	4.28	4.34	4.26	4.15	4.26	0.05
FCR, g/g	4.12	4.09	4.00	4.15	4.21	4.13	0.05
ALT, IU/I	21.6^{b}	28.3^{a}	26.6^{ab}	28.8^{a}	26.8^{ab}	22.4^{b}	0.74
AST, IU/I	165	182	156	161	166	180	4.32
CK, IU/I	1289	1099	1580	1461	1342	1194	69.6
AP, IU/I	589	501	591	514	502	557	22.8
LDH, IU/I	1296^{b}	1846^{a}	1497^{ab}	1393^{ab}	1559^{ab}	1390^{ab}	67.2
Protein, g/dl	4.04^{b}	5.56^{a}	5.25^{a}	5.05^{a}	5.49^{a}	5.06^{a}	0.10
CRP, mg/dl	19.41^{c}	26.37^{a}	23.35^{ab}	20.70^{b}	23.01^{ab}	22.50^{ab}	0.61
LYSE, Ug/dl	3.56^{c}	4.53^{a}	4.67^{a}	4.70^{a}	3.96^{abc}	4.31^{ab}	0.12
PHAG, %	83.3^{a}	83.3^{a}	66.7^{c}	81.7^{ab}	73.3^{bc}	73.3^{bc}	1.46
PKA	0.218	0.172	0.219	0.214	0.213	0.215	0.01
BAS	14.7^{a}	13.4^{a}	11.0^{b}	13.0^{a}	13.4^{a}	12.8^{ab}	0.20

[1]IBW, initial body mass; FBW, final body mass; DI, diet intake; BWG, body mass gain; FCR, feed efficiency ratio: g diet intake/g BWG; [2]ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; LDH, lactate dehydratase; CK, creatine kinase; [3]Protein, CRP, ceruloplasmine; LYSE, lysozyme; PHAG, percentage of phagocytic cells; PKA, potential killing; BAS, number of bacterial absorbed per cell. a, b, c, Values in one row having different superscripts are significantly different at $P \leq 0.05$; S.E.M., standard errors of the means (standard deviation for all rats divided by square root of rat number, n = 48).

was determined. The mass of caecal digesta of rats fed diets with tubers of transgenic line NTR2.27 was significantly higher than in the groups Irga wt and R1F.

The lowest hydration of caecal digesta was found in groups R2P and NTR1.16, while the highest one in the group R1F. The relatively content of dry matter per caecum was similar in these groups. The highest amount of dry matter in the caecum was determined in the group NTR2.27. The caecal pH in the group NTR1.16 was higher, especially compared to non-transgenic Irga wt No significant differences were observed for the activities of α- and β-glucosidase, α- and β-galactosidase and β-glucuronidase in the caecal digesta of rats fed diets containing tubers of the Irga cultivars and the transgenic lines. The highest production of total SCFA as well as of the major acids (acetic, propionic and butyric) was found in the caecum of rats fed diet with tubers of transgenic line NTR2.27. Lower values of SCFA content were observed especially in the groups R1F and R2P.

Table 3. Caecal parameters, bacterial enzyme activity and production of short chain fatty acids (SCFA) in caecum of rats (Juśkiewicz *et al.*, 2004, Zduńczyk *et al.*, 2005b)

	Irga	Irga wt	Transgenic line				SEM
			R1F	R2P	NTR1.16	NTR2.27	
Caecal parameters[1]							
Empty caecum, g	0.28	0.29	0.27	0.30	0.28	0.28	0.01
Caecum content, g	0.98^{ab}	0.90^{b}	0.87^{b}	0.95^{ab}	0.99^{ab}	1.09^{a}	0.02
Dry mater, %	18.6^{ab}	18.7^{ab}	20.5^{a}	17.6^{b}	18.0^{b}	19.3^{ab}	0.29
Dry mater, g	0.178^{b}	0.171^{b}	0.179^{b}	0.170^{b}	0.178^{b}	0.204^{a}	0.01
pH	6.99^{ab}	6.89^{b}	7.03^{ab}	7.08^{ab}	7.21^{a}	6.99^{ab}	0.04
Enzyme activity, U/g							
α-Glucosidase	1.48	1.84	1.66	1.95	1.92	1.80	0.08
β-Glucosidase	0.34	0.45	0.46	0.37	0.39	0.45	0.02
α-Galactosidase	0.80^{b}	1.47^{a}	0.89^{b}	1.12^{ab}	0.91^{b}	0.84^{b}	0.07
β-Galactosidase	3.85^{b}	5.29^{a}	3.88^{b}	4.85^{ab}	4.29^{ab}	4.35^{ab}	0.21
β-Glucuronidase	0.71	0.68	0.60	0.42	0.59	0.58	0.04
SCFA production[1], μmol							
Total	64.75^{b}	64.45^{b}	52.78^{cd}	50.40^{cd}	58.91^{bc}	73.92^{a}	2.23
Acetate	46.68^{ab}	47.23^{ab}	38.03^{bc}	36.39^{c}	42.22^{b}	53.46^{a}	1.62
Propionate	8.65	7.78	7.30	7.08	8.40	9.49	0.34
Isobutyrate	0.79^{ab}	0.85^{ab}	0.82^{ab}	0.62^{b}	0.77^{ab}	0.97^{a}	0.03
Butyrate	6.34^{ab}	6.16^{ab}	4.75^{c}	4.51^{c}	5.27^{bc}	7.22^{a}	0.28
Isovalerate	0.91	0.88	0.87	0.74	0.88	1.02	0.04
Valerate	1.37^{b}	1.56^{ab}	1.02^{c}	1.05^{c}	1.38^{b}	1.75^{a}	0.06

[1]Calculated to 100 g body mass of rats. a, b, c, d, Values in one row having different superscripts are significantly different at $P \leq 0.05$.

DISCUSSION

The results obtained in the present study indicate that genetic modification of potato in order to improve their resistance to a necrotic strain of *Potato virus Y* (PVY^{N}) had no effect on the chemical composition (e.g., crude protein, starch, dietary fibre content and amino-acid composition of protein) and nutritional properties of tubers (diet intake and animal growth). In our original paper (Zduńczyk *et al.*, 2005a; 2005b), we described in details that greater differences between chemical composition (especially in crude protein and starch content) and biological response of rats were observed between tubers from conventional potato cultivars Irga, Ania and Maryna. The obtained results are confirmed by other researchers who worked with plants with genetically improved tolerance to herbicides and plants resistant to diseases. There were no differences in chemical composition between genetically modified potatoes and their conventional ancestor lines (Hashimoto *et al.*, 1999a; 1999b; Rogan *et al.*, 2000). Results of *in vivo* experiments of the above-mentioned authors indicated that tubers of transgenic clones with improved virus- or insect-resistance were nutritionally equivalent to their conventional counterparts. In our study, the chemical composition of tubers and physiological response of rats to experimental diets indicate that transgenic potatoes with genetically-improved resistance to PVY^{N} may be substantially and nutritionally equivalent to the non-transgenic cultivar. However, the results obtained are insufficient to explain some deviations, e.g., the higher content of essential amino acids in tubers of line R2P, the higher enzymatic activity in the caecal content of rats fed a diet with tubers of somaclone Irga, and especially the increased bulk of caecal digesta and higher production SCFA in the caecum of rats fed diet with tubers of the NTR2.27 line. For that reason, a long-term feeding study is necessary to confirm the nutritional properties of tubers of the transgenic lines. A long-term feeding study should allow showing, that genetic transformation of potato (with a truncated gene coding PVY^{N} polymerase or non-translated regions of the PVY^{N} genome, both in sense and antisense orientation), is acceptable with regard to the nutritional and physiological properties of tubers.

CONCLUSION

Concerning the analysed parameters of the chemical composition of tubers and the physiological response of rats to experimental diets, tubers of transgenic line R1F (truncated gene coding for PVY^{N} polymerase in sense orientation), R2P (truncated gene coding for PVY^{N} polymerase in antisense orientation), and NTR1.16 (non-translated regions of PVY^{N} genome in sense orientation) were similar to tubers of the conventional cultivar Irga. Tubers of transgenic line NTR2.27 (non-translated regions of PVY^{N} genome in antisense orientation) increased the

bulk of caecal digesta and the production of SCFA as compared to tubers of the conventional cultivar and the other transgenic clones. Taking into account some deviations, e.g., the higher content of essential amino acids in tubers of line R2.P, higher enzymatic activity in caecal digesta of rats fed a diet with tubers of somaclone Irga, and especially the increased amount of caecal digesta and higher production SCFA in the caecum of rats fed a diet with tubers of line NTR2.27, it seems reasonable to undertake a long-term feeding study to confirm the nutritional properties of tubers of transgenic lines.

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