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The influence of modified 14-3-3 protein synthesis in potato plants on the nutritional value of the tubers

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Abstract

The recently created six transgenic potato genotypes with overexpressed or underexpressed P14-3-3a (29G) and P14-3-3c (20R) isoforms of 14-3-3 protein were field-trialled (1998–2001). The contents of protein, starch, reducing sugars, sucrose and lipids were determined in the transgenic and control tubers harvested from the field. The obtained results showed a significant increase in crude protein content in potatoes with repression of P14-3-3c isoform and in potatoes with blocked P14-3-3a synthesis in comparison to the control line. A stable increase in lipid content of potatoes with overexpression of 14-3-3 protein from *Cucurbita pepo* in the field trials was observed. The variability of the investigated genotypes, in respect to the nutritional components, was statistically analysed using discriminant function and cluster analyses. The dominant influence of the variability of the genotypes exerted significant differentiation of protein, lipid and starch contents. These components showed the greatest discriminant power in the variability of genotypes. These results confirm the suggestion that 14-3-3 protein co-ordinates primary metabolite synthesis in plants.

Keywords: Transgenic potato; Nutritional value; Protein 14-3-3

1. Introduction

Genetic transformation of plants is the modern method that facilitates the controlled and fast development of new properties of plants, in terms of both culture requirements and chemical composition. Nowadays, there are high expectations concerning genetic modifications of plants with changed expression of regulatory genes and those co-ordinating whole metabolic pathways. Results of in vitro and in vivo studies on the protein 14-3-3 suggest that this protein fulfils the role of such a co-ordinator in plants (Aitken, 1996; Szopa, Wróbel, Matysiak-Kata, & Świędrych, 2001; Wilczyński, Kulma, & Szopa, 1998). This protein is widely distributed in nature. Besides plants, it is present in mammals, insects and fungi (for review see Wilczyński, Kulma, Markiewicz, & Szopa, 1997). In plant tissues it is proposed, that this protein has regulatory functions, for example in the process of nitrogen fixation and carbohydrate metabolism, via direct interaction with nitrate reductase and sucrose phosphate synthase, respectively (Bachmann, Huber, Liao, Gage, & Huber, 1996; Szopa et al., 2001). Moreover, the cultivation experiments conducted on model plants *Arabidopsis thaliana*, and on tobacco with modified levels of the protein 14-3-3 synthesis, showed its involvement in adaptation of the plant to environmental conditions such as cold and salinity (Chen et al., 1994; Jarillo, Capel, Leyva, Martinez-Zapater, & Salinas, 1994).

Recently, six isoforms of the protein 14-3-3 were identified and isolated from potato (Wilczyński, Kulma, & Szopa, 1998). Potato plants (*Solanum tuberosum*, cv. Desiree) were genetically transformed and transgenic plants with overexpression of heterologous gene 14-3-3 of pumpkin (*Cucurbita pepo* var.*patissonina*) and plants with blocked synthesis of isoforms *a* and *b* of the protein 14-3-3 (antisense transformations) were obtained. Studies on the raised transgenic lines of potatoes showed that proteins belonging to the 14-3-3 family participate in the vegetative cycle of the plants and have an influence on the synthesis of catecholamines. It is

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proposed that the 14-3-3 protein indirectly modifies the level of carbohydrates in potato plants via regulation of catecholamine synthesis (Szopa, 2002; Świędrych, Prescha, Matysiak-Kata, Biernat, & Szopa, 2002; Wilczyński, Kulma, Feiga, Wenczel, & Szopa, 1998).

The objective of this work was to estimate an influence of the modified 14-3-3 protein synthesis in the potato plant on the contents of the nutritive components: protein, starch, reducing sugars, sucrose and lipids in the tubers from the field trials of 1998–2001. The variability in respect of the nutritional components in the investigated potato genotypes during the 4 years of field trials, was statistically assessed the methods of discriminant function and cluster analyses. The presented results are a part of a research project, which includes the assessments of nutritional value of transgenic potatoes with various levels of the protein 14-3-3 synthesis and their usefulness in food processing.

2. Material

2.1. Materials

The potato plants (*Solanum tuberosum* L. cv. Desiree) were genetically transformed as described previously (Szopa & Muller-Rober, 1994; Szopa et al., 2001; Wilczyński, Kulma, & Szopa, 1998a) and potato lines of six transgenic types with various rates of 14-3-3 protein isoforms synthesis were obtained (Table 1). The transgenic plants were preselected by PCR and Western analysis as recently described (Wilczyński, Kulma, & Szopa, 1998) and three to five transgenic lines of each transgenic type (with the lowest or highest 14-3-3 isoforms quantity, respectively) were chosen for further cultivation. The plants were grown in the greenhouse in soil under a 16 h light (22 °C)/8 h dark (15 °C) regime. Tubers from the greenhouse were propagated in a field; the field experiment was performed from April to September of 1998–2001 in the vicinity of Wrocław, Poland. After harvesting of mature tubers from 75 plants of each transgenic line, the mean samples (3 kg), representing the size distribution of the whole batches, were collected for analysis. For analyses of the control line Desiree, three to five mean samples (1 kg) from the whole batch were collected. The tubers were washed, wiped dry and peeled (the thickness of peel was 1–2 mm), then cut into 1 cm slices. The samples were freeze-dried and fine-ground in a mill.

2.2. Methods

2.2.1. Determination of crude protein

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

2.2.2. Determination of starch

The samples of tubers were extracted with 80% ethanol–50 mM HEPES–KOH, pH 7.4 at 80 °C, the supernatants were removed and the remains were homogenised in 0.2 M KOH, and following incubation at 95 °C, adjusted to pH 5.5 with 1 M acetic acid. Starch was hydrolysed with amyloglucosidase, and the released glucose content was determined enzymatically using Test-Combination Starch (Boehringer Mannheim, Germany). At least three measurements were performed for each sample.

2.2.3. Determination of reducing sugar and sucrose contents

The contents of reducing sugars and sucrose—after hydrolysis to reducing sugars (5 min at 70 °C in 2.8% HCl)—were determined in the potato samples by the

Table 1
The characteristics of analysed genetic types of potatoes with modified 14-3-3 protein synthesis

Genetic type	Characteristics
Desiree	Control line
J2	Overexpression of the 14-3-3 protein from *Cucurbita pepo* var. *patissonina* with high homology to potato isoform P14-3-3c (previously called 20R) of 14-3-3 protein
J4	Repression of the isoform P14-3-3c of the 14-3-3 protein obtained by antisense technique
J5	Repression of the isoform P14-3-3a (previously called 29G) of the 14-3-3 protein obtained by antisense technique
G1	Repression of both P14-3-3c and P14-3-3a isoforms obtained by antisense technique
J1	Repression of the ADP-ribosylation factor which causes the increase in P14-3-3a isoform content
G2	Repression of both ADP-ribosylation factor and P14-3-3a isoform, which causes the 14-3-3 protein synthesis similar to the control plants

Nizovkin- Jemieljanova method (Leszczyński & Lisińska, 1972). At least three measurements were performed for each sample.

2.2.4. Determination of lipid content

Total (raw) lipids were determined in the potato samples using the extraction-gravimetrical method. Extraction was performed using the Bligh–Dyer (1959) method. The solvent from chloroform extracts was evaporated under nitrogen and the solid remains were weighed after drying at 105 °C. At least two measurements were performed for each sample.

2.2.5. Statistical analysis

Statistical calculations were done with the *t*-test. The term "significant" is used when $P < 0.05$ with the *t*-test. The multiple variability of the transgenic types and control line of potatoes was analysed by methods of discriminant function and cluster analyses using the statistical data analysis program STATISTICA 5.1. (StatSoft Polska, Poland).

3. Results and discussion

3.1. General

The results of the estimations of protein, starch, reducing sugars, sucrose and lipids in potatoes from 4 consecutive years of field experiment are presented in Table 2. All results were calculated in relation to the dry weight of potato tubers. Differences in the contents of these components were observed both between the genotypes of potato and between the years of field trials.

3.1.1. The protein level

The total protein contents in tubers from control plants harvested in the investigated years ranged from 8.55 to 12.13% of dry weight (DW). The lowest differences in the amounts of protein occurred during these years in potatoes J1 with overexpression of P14-3-3a isoform (10.7–12.1% DW). The data show a significant increase in the crude protein value for tubers from the transgenic type with repression of P14-3-3c isoform (J4) in comparison to the control line (from 13 to 21%) within the 4-year field experiments. These results confirm the suggestion that protein 14-3-3 is involved in vivo in regulation of nitrate reductase activity and, consequently, in amino acid and protein synthesis (lately reported by Święcrych et al., 2002). The potatoes J1 were distinguished in 3 years of field trials, except 1999, by the significantly higher protein contents which ranged from 21% in 1998 even to 38% in 2001, in comparison to Desiree. The lack of consistency in all investigated years does not allow any conclusive explanation of these results; however, it suggests that both,

Table 2
The protein, starch, reducing sugar, sucrose and lipid contents in the tubers of control and transgenic potatoes from the field trials 1998–2001

	1998		1999		2000		2001	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Protein (g/100 g DW)								
Desiree	9.78	0.274	12.1	1.31	8.55	0.715	8.79	0.537
J2	11.0	1.051	12.5	0.862	10.0*	0.333	10.5*	1.00
J4	11.8*	0.881	14.4*	0.740	10.4*	0.607	9.94*	0.705
J5	10.0	0.702	13.1*	0.378	9.73	1.05	9.55	1.28
G1	11.3*	0.787	12.8	0.514	9.24	0.903	9.28	1.55
J1	11.9*	0.871	11.8	0.909	10.7*	0.834	12.1*	1.13
G2	9.91	0.630	13.9*	1.04	11.9*	0.880	9.87	0.307
Starch (g/100 g DW)								
Desiree	66.4	5.30	69.4	5.53	75.5	4.09	75.0	1.83
J2	64.1	4.82	68.7	4.27	80.5	3.52	78.3*	1.55
J4	70.1	5.01	71.9	4.49	77.4	4.92	77.2	3.69
J5	70.0	7.43	69.3	5.70	78.8	4.79	76.8	2.51
G1	70.7	5.22	72.4	6.76	80.1	4.44	78.1	2.02
J1	70.4	4.79	72.5	7.08	75.8	4.90	75.8	1.98
G2	75.3*	6.39	69.2	4.37	74.5	5.25	81.3	4.09
Reducing sugars (g/100 g DW)								
Desiree	1.71	0.4972	0.62	0.0714	0.63	0.0624	1.33	0.661
J2	2.08	1.4464	0.82*	0.0960	0.54	0.2412	0.80	0.345
J4	0.87*	0.2555	0.72	0.1967	0.49	0.1190	0.76	0.224
J5	0.98*	0.3989	0.61	0.0726	0.50	0.1132	0.95	0.295
G1	1.91	0.6993	0.91	0.2261	0.66	0.2988	0.96	0.253
J1	1.08	0.5398	0.52	0.0666	0.93*	0.1030	0.63	0.143
G2	0.89	0.4142	1.23*	0.1865	0.63	0.1301	1.63	0.452
Sucrose (g/100 g DW)								
Desiree	1.53	0.6651	0.67	0.0794	1.47	0.1701	1.04	0.111
J2	1.02	0.2442	0.87	0.1997	1.19*	0.1465	0.73*	0.128
J4	1.18	0.2827	0.54	0.0742	1.32	0.1122	0.79*	0.083
J5	1.24	0.3706	0.66	0.0877	1.29	0.3509	0.78	0.283
G1	1.11	0.2087	0.53	0.0770	1.28	0.3806	0.81*	0.103
J1	1.19	0.1860	1.10*	0.2090	0.96*	0.1103	0.73	0.255
G2	1.08	0.3351	1.22*	0.1863	1.38	0.2401	1.06	0.199
Lipids (mg/100 g DW)								
Desiree	0.52	0.0321	0.58	0.0208	0.41	0.0404	0.41	0.0153
J2	0.88*	0.0252	0.63*	0.0115	0.45	0.0245	0.52*	0.0451
J4	0.54	0.0306	0.56	0.0300	0.41	0.0403	0.48	0.0534
J5	0.56	0.0404	0.60	0.0252	0.41	0.0207	0.43	0.0619
G1	0.72*	0.0265	0.62	0.0153	0.43	0.0321	0.45	0.0573
J1	0.56	0.0208	0.55	0.0153	0.36	0.0153	0.44*	0.0153
G2	0.46	0.0346	0.59	0.0252	0.45	0.0586	0.36	0.0265

S.D., standard deviation; DW, dry weight.

* Significant differences in comparison with the control line in the individual years of field trials.

repression of the P14-3-3c isoform and increase in P14-3-3a isoform level in the plant, induces a higher protein synthesis. It is thus speculated that the level of protein synthesis in the plant may depend on a ratio between the 14-3-3 isoforms.

3.1.2. The carbohydrate contents

The direct interaction of the 14-3-3 protein with sucrose phosphate synthase and the influence on the catecholamine and soluble sugar contents in leaves and tubers of the greenhouse-grown potato plants with modified 14-3-3 synthesis indicated the regulatory function of 14-3-3 in carbohydrate metabolism of the plants. Of special interest was the question of how stable are the changes in carbohydrate contents in the field-trialled transgenic tubers.

The mean contents of starch in tubers of the analysed genotypes ranged between 64.1 and 81.3% DW. A relatively high stability of the mean starch contents (70.4–75.8% DW) was observed in the potatoes J1 in the 4-year experiment. In comparison to the control line, differences in starch accumulation of transgenic lines were not significant between the individual years of field trials. The high variability of the starch content in the analysed genotypes has been confirmed by the high values of the standard deviation of results, especially during first two years of field experiments. The obtained results for reducing sugars show that the amounts of this component varied in the consecutive years of cultivation. The highest contents of reducing sugars were observed in tubers harvested in 1998 (0.87–2.08% DW) and the lowest in 2000 (0.49–0.93% DW). The mean sucrose contents of the analysed tubers ranged from 0.53 to 1.53% DW and showed lower differences than reducing sugars between the years of cultivation. None of the studied transgenic genotypes differed significantly from the control line in all four years of field trials in regard to reducing sugars and sucrose levels in tubers.

3.1.3. The lipid contents

An increase of the total lipid contents in the transgenic potato tubers with overexpression of the P14-3-3c synthesis, up to 69% in comparison to the control group, in field trials of 1998–2000 was recently reported (Prescha, Biernat, & Szopa, 2002; Prescha, Świędrych, Biernat, & Szopa, 2001). The gathering of the data from 4 years of field trials confirms these changes (Table 2). However the differences in lipid contents of transgenic potatoes J2 from 1999 and 2000 were slight (in 2000 not significant, the result of *t*-test 1.78).

3.1.4. Statistical analysis of variability

In order to determine the multiple variability of the investigated transgenic types in regard to the contents of nutritional components in 4 years of cultivation, we have used the discriminant function analysis and the cluster analysis method of the "furthest neighbours" (Caliński, Dyczkowski, & Sitek 1979; Mądry, 1993; Morrison, 1990).

The multidimensional analysis of variance, MANOVA, has shown a significant differentiation of the transgenic types, which was proved by the low value of the Wilks' lambda coefficient (Table 3).

In order to assess the multi-feature similarity of the investigated objects we have employed a dendrogram tree by means of the "furthest neighbours" method (Mądry, 1993), with the determination of differences between the objects in the form of the F statistic after a formula:

$$F_\alpha = \frac{\alpha}{\left(\frac{a}{2}\right)} |p| n - a - b - p + 2$$

where n is a number of observations, p is a number of investigated traits, α is a significance level, a is a number of objects and b is a number of years.

The obtained dendrogram (Fig. 1) has been bisected by a line placed on the critical value's level $F_\alpha = 5.98$. The investigated transgenic types and control line, hanging on the horizontal line, have formed four homogeneous groups, which differed considerably in regard to the protein, starch, reducing sugar, sucrose and lipid contents. The nearer the position of each homogeneous group, the greater was the similarity between the groups of the investigated genotypes. The transgenic type J1 and the types with the repression of 14-3-3 isoforms: J5, G1 and J4, were distinguished by a substantial similarity in terms of the investigated traits and formed the four-element homogeneous group. From this group the type J1 emerged, which could have formed a one-element group. However, the Euclidean distance between the type J1 and the repressed types J5, G1 and J4 was near the critical value F_α. The remaining genotypes showed a considerable variability in the contents of nutritional components. Desiree and G2 showed the longest Euclidean distance, whereas J1 and J4 as well as G2 and J5 showed considerable similarity between groups.

The variability of the investigated genotypes has also been estimated by means of the Mahalanobis distance, which is a measure of distance between two objects as a sum of squared differences of mean values of the investigated traits for the objects. This distance is expressed in the units of standard deviation of the error with

Table 3
The multivariate analysis MANOVA of six transgenic types in respect to the contents of nutritional components

Wilks' lambda	*R* Rao	df1	df 2	*P*-level
0.1124	1.6961	30	70	0.0316

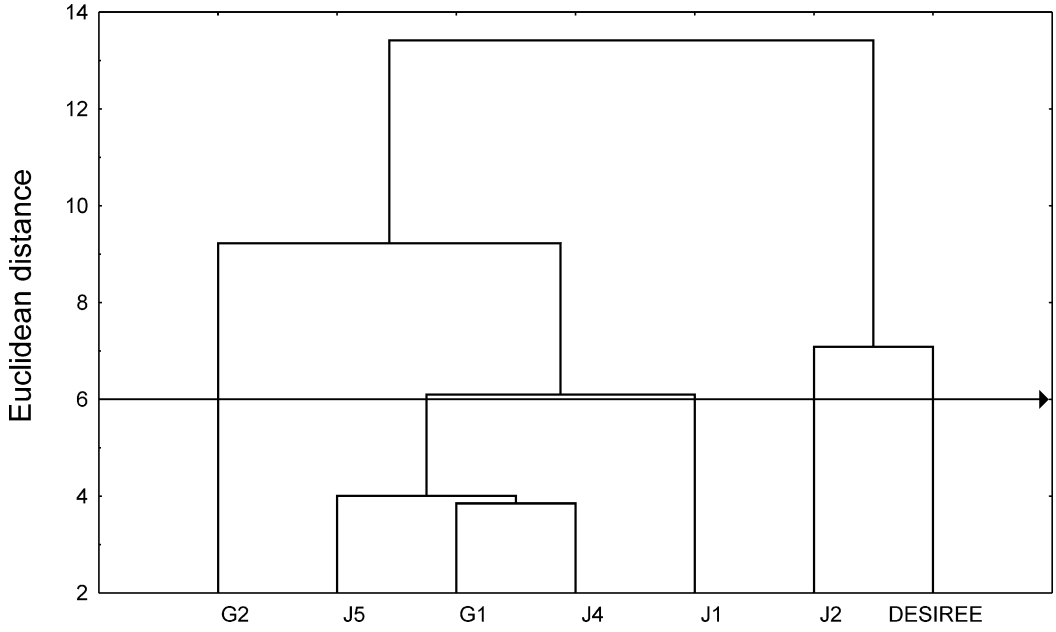

Fig. 1. Similarity of the genotypes in respect to the investigated traits.

Table 4
The squared Mahalanobis distances between the analysed genotypes

Genotypes	Desiree	J2	J4	J5	G1	J1	G2
Desiree	0	21.31**	9.61*	4.85	20.17**	6.81	15.64**
J2	21.31**	0	7.33	7.40	0.99	12.15	14.68**
J4	9.61	7.33	0	2.04	5.77	0.82	4.52
J5	4.85	7.41	2.04	0	7.12	2.81	10.19*
G1	20.17**	0.99	5.77	7.12	0	9.71	10.12*
J1	6.81	12.15*	0.82	2.81	9.71*	0	3.71
G2	15.64**	14.68*	4.52	10.19*	10.12*	3.71	0

* Significant differences $\alpha = 0.05$.
** Significant differences $\alpha = 0.01$.

Table 5
The gathering of discriminant function analysis in regard to the investigated traits

Traits	Wilks' lambda	Partial Wilks' lambda	*F*	*P*-level
Protein	0.231	0.485	3.000	0.034*
Starch	0.307	0.365	4.912	0.004**
Sucrose	0.142	0.788	0.760	0.610
Reducing sugars	0.161	0.699	1.215	0.346
Lipids	0.308	0.365	4.929	0.004**

* Significant differences $\alpha = 0.05$.
** Significant differences $\alpha = 0.01$.

simultaneous regard to the error covariance between the traits (Table 4).

After analysing the data obtained in Table 4, we can conclude that the genotypes J2–Desiree, G1–Desiree, G2–Desiree showed a large distance between them in the investigated multidimensional space. The significant distances between analysed objects in the space formed by the five investigated nutritional components agreed highly with the results of the cluster analyses. The dominant influence of the variability of the genotypes caused significant differentiation of protein, lipid and starch contents, whereas the remaining analysed components showed no considerable deviates in the investigated population of genotypes (Table 5).

Table 6 shows the multiple differentiation between the groups of homogeneous objects in the space of the canonical variables. To interpret the meaning of the canonical variables we used both the standardised canonical coefficients (Roots 1–5) and the correlation coefficients between the mean contents of the nutritional components for the objects and the canonical variables. The first canonical variable ensured 60.5% of the multiple variability of the objects. The greatest contributions in the creation of this variable were starch and lipids. The large discriminatory power of these traits also confirmed the scores in Table 5, where the mentioned components were distinguished among the analysed variables by the

Table 6
The values of the standardised coefficients for the canonical variables

Variable	Root 1	Root 2	Root 3	Root 4	Root 5
Protein	0.313	−1.876	−0.248	0.301	0.223
Starch	2.695	−1.151	0.141	−0.279	−0.560
Sucrose	0.224	−0.724	0.275	0.709	−0.708
Reducing sugars	0.038	−0.919	0.895	−0.285	0.565
Lipids	2.590	0.714	−0.600	0.518	−0.463
Eigenvalue	2.195	1.149	0.242	0.033	0.009
Cumulated contribution	0.605	0.922	0.988	0.997	1.000

Table 7
Factor matrix. Correlation: variables—canonical roots

Variable	Root 1	Root 2	Root 3	Root 4	Root 5
Protein	0.080	0.612	−0.537	0.492	−0.296
Starch	0.778	0.593	−0.054	0.096	−0.172
Sucrose	−0.176	−0.108	0.491	0.444	−0.720
Reducing sugars	0.0807	−0.006	0.829	0.171	0.525
Lipids	0.709	0.224	0.671	0.290	0.598

highest values of F statistic. The relevance of these traits to the first canonical variable confirmed the high correlation between the groups (Table 7).

The second canonical variable is responsible for 31.6% of the multidimensional variability between the objects. The dominant influence of this variable was on protein and starch formation. The correlation of protein and starch with the second canonical variable was also high. The remaining canonical variables contributed only very slightly to the creation of the multiple variability of genotypes.

The obtained results of canonical variable analysis show that the greatest influence on the studied variability was on protein, starch and lipids. These traits also led to the comparatively high values of the F statistic. Low standardised canonical coefficients and correlation coefficients show that the smallest discriminatory powers in the population of analysed genotypes were reducing sugars and sucrose.

In the investigated population, the genotypes Desiree and G2 differed considerably from the remaining genotypes in regard to the protein, sucrose, reducing sugar, starch and lipid contents.

4. Conclusions

The proposed regulatory functions of the protein 14-3-3 were in plant metabolism demonstrated, as described, by the directed changes in the metabolite levels in greenhouse-grown plants with modified synthesis of this protein. The 4-year field experiment, using the transgenic potatoes with modified synthesis of 14-3-3 isoforms, has shown that the changed levels of 14-3-3 influenced the accumulation of nutritional components in tubers. The highest stability of these changes in seasons of cultivation was shown only for protein and lipid contents. Statistical analysis of variability demonstrated significant differentiation of transgenic genotypes in regard to the contents of nutritive components in the studied potato tubers. The greatest discriminant power in the variability of investigated genotypes was shown by protein, starch and lipids. These results confirm the suggestion that 14-3-3 coordinates the primary metabolite synthesis and that modulatory function of 14-3-3 in starch metabolism seems to be dependent on environmental factors.

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References

Aitken, A. (1996). 14-3-3 and its possible role in co-ordinating multiple signalling pathways. *Trends in Cell Biology*, *6*, 340–347.

AOAC. (1990). *Official methods of analysis* (15th ed.). Arlington, VA: Association Official Analytical Chemists.

Bachmann, M., Huber, J. L., Liao, P. L., Gage, D. A., & Huber, S. C. (1996). 14-3-3 proteins associate with the regulatory phosphorylation site of spinach leaf nitrate reductase in an isoform-specific manner and reduce dephosphorylation of Ser-543 by endogenous protein phosphatases. *FEBS Letters*, *398*, 127–131.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*, *37*, 911.

Caliński, T., Dyczkowski, A., & Sitek, M. (1979). Procedury testów jednoczesnych w wielozmiennej analizie wariancji. *Matematyka stosowana*, *14*, 5–31.

Chen, Z., Fu, H., Liu, D., Chang, P. F. L., Narasiman, M., Feri, R., Hasegawa, P. M., & Bressan, R. A. (1994). A NaCl-regulated plant gene encoding a brain protein homolog that activates ADP ribosyltrasferase and inhibits protein kinase C. *Plant J.*, *6*, 729–740.

Jarillo, J. A., Capel, J., Leyva, A., Martinez-Zapater, J. M., & Salinas, J. (1994). Two related low-temperature - inducible genes of Arabidopsis encode proteins showing high homology to 14-3-3 proteins, a family of putative kinase regulators. *Plant Mol. Biol.*, *25*, 693–704.

Leszczyński, W., & Lisińska, G. (1972). Metody badania jakoœci bulw traktowanego w uprawie herbicydami ziemniaka jako surowca dla przemysłu spo¿ywczego. *Prace Naukowe Instytutu Technologii Organicznych Tworzyw Sztucznych Politechniki Wrocł*awskiej, *9*, 55–61.

Mądry, W. (1993). *Studia statystyczne nad wielowymiarową oceną zróżnicowania cech ilościowych w kolekcjach zasobów genowych zbóż*. Warszawa: Wydawnictwo SGGW.

Morrison, D. F. (1990). *Wielocechowa analiza statystyczna*. Warszawa: PWN.

Prescha, A., Świędrych, A., Biernat, J., & Szopa, J. (2001). The increase in lipid content in potato tubers modified by 14-3-3 gene overexpression. *Journal of Agricultural Food Chemistry*, *49*, 3638–3643.

Prescha, A., Biernat, J., & Szopa, J. (2002). Quantitative and qualitative analysis of lipids in genetically modified potato tubers with varying rates of 14-3-3 protein synthesis. *Nahrung/Food*, *46*(3), 179–183.

Świędrych, A., Prescha, A., Matysiak-Kata, I., Biernat, J., & Szopa, J. (2002). Repression of the 14-3-3 gene affects the amino acid and mineral composition of potato tubers. *Journal of Agriculture and Food Chemistry*, *50*, 2137–2141.

Szopa, J. (2002). Transgenic 14-3-3 isoforms in plants: the metabolite profiling of repressed 14-3-3 protein synthesis in transgenic potato plants. *Biochemical Society Transactions*, *30*, 405–410.

Szopa, J., & Muller-Rober, B. (1994). Cloning and expression analysis of an ADP-ribosylation factor from Solanum tuberosum L. *Plant Cell Reports*, *14*, 180–183.

Szopa, J., Wróbel, M., Matysiak-Kata, I., & Œwiêdrych, A. (2001). The metabolic profile of the 14-3-3 repressed transgenic potato tubers. *Plant Science*, *161*, 1075–1082.

Wilczyński, G., Kulma, A., Markiewicz, E., & Szopa, J. (1997). Characterisation of the role of the 14-3-3 protein family in potato plants. *Cellular and Molecular Biology Letters*, *2*, 239–241.

Wilczyński, G., Kulma, A., & Szopa, J. (1998). The expression of 14-3-3 isoforms in potato is developmentaly regulated. *J. Plant Physiol*, *153*, 118–126.

Wilczyński, G., Kulma, A., Feiga, I., Wenczel, A., & Szopa, J. (1998). Manipulating of 14-3-3 protein expression results in the changes of catecholamine content in potato plants. *Cellular and Molecular Biology and Letters*, *3*, 75–91.