Increase in Lipid Content in Potato Tubers Modified by 14-3-3 Gene Overexpression

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Recently, transgenic potato plants were created with overexpression of the 14-3-3 protein derived from *Cucurbita pepo*. Detailed analysis of those plants suggested that the function of the isolated 14-3-3 isoform is in the control of carbohydrate and lipid metabolism in the plants. 14-3-3 protein overexpression gave rise to an increase in soluble sugar and catecholamine contents in both leaves and tubers. It is proposed that 14-3-3 protein affects carbohydrate metabolism in potato plants via regulation of catecholamine synthesis. Furthermore, genetically modified potato tubers with 14-3-3 protein overexpression showed changes in lipid content and composition. The transgenic potato tubers contained 69% more total fat compared to the wild-type plant. Separation of tuber lipids into polar and nonpolar fractions revealed that the transgenic potato tubers contained almost 3 times more nonpolar lipids than the control. Analysis of fatty acid composition, conducted by the means of gas chromatography, showed that linoleic acid was the main fatty acid present in the tubers of both modified and control potato plants. In the nonpolar fraction of the fat of the transgenic tubers the unsaturated fatty acids exhibited a higher participation in the sum of all fatty acids.

Keywords: *14-3-3 protein; transgenic plant; catecholamines; carbohydrate metabolism; lipid analysis; Solanum tuberosum*

INTRODUCTION

The development of plant transformation techniques in the past decade has positively affected plant biotechnology and thus food production. Almost all agriculturally important plants can be transformed and thus modified. The potato is among those plants that are most extensively engineered; their manipulation is mainly concerned with carbohydrate metabolism. Carbohydrates are the compounds accumulated in storage organs and are the main component of our diet. In addition, carbohydrates provide a carbon skeleton for the synthesis of amino acids, nucleotides, and other organic compounds.

So far, manipulations have been reported for the Calvin cycle enzymes, the enzymes involved in starch biosynthesis in photosynthetic tissues and storage organs, the enzymes of the major route for channeling photoassimilates into the cytosol, and the enzymes active in the translocation of photoassimilates from source to sink tissues in transgenic potato plants (for reviews see refs *1* and *2*). The results of these experiments show that plant metabolism can be changed through genetic engineering. The question remains whether such a change can be specifically directed to alter the quantity of metabolites in a desired way. The response of plant carbohydrate metabolism to the genetic engineering of specific enzymes varies from the predictable to the unexpected. This is due to the existence of alternative metabolic routes within the same tissue.

In the case of a complex metabolic pathway such as carbohydrate synthesis, it is often impossible to manipulate the metabolism in the desired way by changing a single enzyme. There are usually alternative pathways by which the missing enzyme is compensated for. An alternative way of changing the metabolic pathway is to affect a given metabolic step indirectly by adding or removing the protein influencing the activity of the target enzymes. A potential candidate for such a manipulation is the 14-3-3 protein, functioning as an adapter protein for several enzymes such as sucrose phosphate synthase and nitrate reductase.

The first 14-3-3 protein was isolated from bovine brain tissue as an abundant, acidic, brain-specific polypeptide. Since this discovery several different functions have been proposed for this class of protein. These proteins are highly conservative and are found in a broad range of organisms including mammals, insects, yeasts, and plants (for reviews see refs *3* and *4*).

Many recent findings indicate the participation of these proteins in cell cycle control and gene expression. Members of the 14-3-3 family activate neurotransmitter synthesis, activate ADP-ribosylation of proteins, regulate protein kinase C and nitrate reductase, display phospholipase A_2 activity, and associate with the products of proto-oncogenes, oncogenes, and the cdc 25 gene (for a review see ref *5*). The broad spectrum of activities that are affected by 14-3-3 proteins shows their usefulness in the modification of plant metabolism.

The cDNA from *Cucurbita pepo* var. *patissonina* has previously been isolated and sequenced (*6*). The nucleotide and deduced amino acid sequences show very high

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similarity to known 14-3-3 protein sequences from other sources (*7*). An initial characterization of transgenic potato plants overexpressing the *Cucurbita* 14-3-3 cDNA has recently been presented (*8*). A decrease in 14-3-3 protein content, concomitant with an increase in nuclease activity, was detected in senescent plants, and this was markedly delayed in transgenic potato plants that overexpressed the 14-3-3 protein. It was proposed that a function of the isolated 14-3-3 isoform is in the control of nuclease activity and hence senescence.

In this study carbohydrate and lipid contents in the tubers and leaves of transgenic plants grown in a greenhouse and in a field were compared. It has been found that overexpression of the 14-3-3 protein induced an increase in catecholamine and soluble sugar contents in leaves and a reduction in tuber size and starch content in greenhouse plants. It is proposed that the 14-3-3 protein affects carbohydrate metabolism in potato via regulation of catecholamine synthesis. An increase in fat content in transgenic tubers was also observed; however, the mechanism of this alteration is as yet unknown.

MATERIALS AND METHODS

Plant Material and Bacterial Strains. Potato plants (*Solanum tuberosum* L. cv. Desiree) were obtained from Saatzucht Fritz Lange KG (Bad Schwartau, Germany). The plants in the tissue culture were grown under a 16 h light/8 h dark regime on MS medium (*9*) containing 0.8% sucrose. The plants in the greenhouse were cultivated in soil under a 16 h light (22 °C)/8 h dark (15 °C) regime. The plants were grown in individual pots and were watered daily. Tubers were harvested 3 months after the transfer of the tissue culture plants to the greenhouse. Field trials were performed in the vicinity of Wrocław, Poland, between April and September. *Escherichia coli* strain *DH5a* (Bethesda Research Laboratories, Gaithersburg, MD) was cultivated using standard techniques (*10*). *Agrobacterium tumefaciens* strain *C58C1* containing plasmid pGV2260 (*11*) was cultivated in YEB medium (*12*).

Recombinant DNA Techniques. DNA manipulations were performed essentially as described by Sambrook et al. (*10*). DNA restriction and modification enzymes were obtained from Boehringer Mannheim (Mannheim, Germany) and New England Biolabs (Beverly, MA). *E. coli* strains *DH5a* and *XL1-Blue* were used for bacterial work.

Construction of the 14-3-3 Gene. A 1.2 kb *Sma*I-*Asp718* fragment of plasmid A215 (*8*) encoding a 14-3-3 protein from *C. pepo* was ligated in the sense orientation into the *Sma*I site of the plant transformation vector BinAR. The vector was introduced into the *A. tumefaciens* strain *C58Cl:pGV2260* as described (*13*), and the integrity of the plasmid was verified by restriction enzyme analysis.

Transformation of Potato Plant. Young leaves of the wild-type potato *S. tuberosum* L. cv. Desiree were used for transformation with *A. tumefaciens*, by immersing the leaf explants in the bacterial suspension. *A. tumefaciens*-inoculated leaf explants were subsequently transferred to callus induction and shoot regeneration medium (*13*).

Screening of Transgenic Plants. Transgenic potato plants were screened using Northern and Western blot analysis with a 14-3-3 specific cDNA fragment as a probe in the former case and as an antiserum against recombinant 14-3-3 protein in the latter case, as described previously (*2*, *14*).

Protein Extraction. Tissues were powdered in liquid nitrogen and extracted with 50 mM HEPES−NaOH buffer, pH 7.4, containing 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 0.5 mM PMSF, 10% glycerol, 0.1% Triton X-100, and 0.2% 2-mercaptoethanol (buffer E) (*15*). After 20 min of centrifugation at 13000 rpm (HERAEUS minifuge) and 4 °C, the supernatant was used immediately or frozen in liquid nitrogen and stored at −70 °C until use.

Determination of Starch and Soluble Sugars. Potato tuber slices and leaf disks were extracted with 80% ethanol−50 mM HEPES−KOH, pH 7.4, at 80 °C. The supernatant was used for enzymatic analysis of glucose, fructose, and sucrose (*16*). 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 content was determined enzymatically.

Protein Free Extract. Frozen plant tissue was powdered in liquid nitrogen and extracted with 10% TCA. The TCA extract was processed basically as described by Steiner (*17*). TCA supernatant was extracted six times with 10 volumes of ethyl ether. The extracted phase was evaporated in a vacuum.

Catecholamine Assay. The manufacturer's (Bio-Rad) HPLC assay system was used. It measures all three catecholamines (dopamine, norepinephrine, and epinephrine). The method consists of two purification steps and electrochemical detection with the use of a Merck-Hitachi HPLC instrument, model D-7000. In the first step, protein-free extract was chromatographed on a cation exchange column and finally on an analytical reversed phase column type JEC. Both columns are included in the Bio-Rad kit. This method is routinely used in clinical laboratories for catecholamine assay on urine.

Determination of Vitamin C. Vitamin C content in the peeled potato tubers was determined according to the 2,6-dichloroindophenol titrimetric method (*18*).

Lipid Content and Composition. The total fat (raw) in the peeled potato tubers was determined using the extraction−gravimetrical method. Extraction was performed using the Blight and Dyer method (*19*). The chloroform extract was evaporated under nitrogen, and the solid remains were weighed after drying at 105 °C. Extracted fat was fractionated into nonpolar (neutral) and polar fractions using chromatographic columns filled with silica gel. After the sample of fat had been applied on the column, the nonpolar lipids was eluted by chloroform and polar lipids by methanol (*20*). The obtained fractions were quantitatively evaluated by the gravimetrical method, which includes solvent evaporation under nitrogen atmosphere, drying the remaining part at 105 °C, and finally weighing the solid matter.

Analysis of Fatty Acids. For tubers, the fatty acid composition of the total fat from its nonpolar fraction was examined using the gas chromatography method. Methyl esters of the fatty acids (FAMEs) were obtained by means of esterification of fat samples according to the Szymczak method (*21*) with modification. This method is suitable for small lipid quantities with large amounts of unsaponifiables. Fifty milligrams of lipids was saponified at 70 °C for 1 h with 1 mL of 2 M KOH in 75% aqueous methanol. The unsaponifiable material was extracted two times with petroleum ether and then removed. The potassium salts of the fatty acids were treated with 1 mL of 2 M HCl in water (at 70 °C for 30 min). After the addition of saturated sodium chloride solution, the fatty acids were extracted with two 1 mL portions of hexane. The obtained fatty acids were esterified at 70 °C using 1 mL of 0.5 M KOH for 30 min and then 1 mL of 1.25 M HCl for 30 min (both solutions in anhydrous methanol). The FAMEs were extracted with hexane as described previously. The methyl esters mixture was separated on a capillary column, CP-Sil 88 Chrompack (50 m × 0.25 mm). Helium was used as a carrier, and the separation was carried out at a temperature programmed from 150 °C (for 6 min) to 235 °C; the temperature increased at a rate of 6 °C/min. The identification of particular fatty acids was accomplished by comparison with external standards.

RESULTS AND DISCUSSION

Members of the 14-3-3 protein family display several activities, for example, tyrosine and tryptophan hydroxylase activation, regulation of sucrose phosphate synthase, nitrate reductase, protein kinase C, and endonuclease, phospholipase A_2 activity, and binding to

Table 1. Effect of 14-3-3 Manipulation on Tuber Development[a]

	FW per plant (g)	tuber no. per plant	tuber density (g/cm^3)	starch (μmol/ g of FW)	mean FW (g) per tuber
D	64.9 ± 6	7.3 ± 0.6	1.080 ± 0.004	557 ± 45	8.9
J2	66.1 ± 7	10.5 ± 0.7	1.090 ± 0.004	372 ± 45	6.3

[a] Transgenes with overexpression (J2) of 14-3-3 protein synthesis were analyzed and compared to the control (D). Data were obtained from four independent transgenic lines (J2.51, J2.52, J2.53, and J2.54), and each line was represented by at least six plants.

Table 2. Carbohydrate Partitioning in the Leaves of Control (D) and Transgenic Potato Plants Overexpressing *C. pepo* 14-3-3 Protein (J2) (Micromoles per Gram of FW)[a]

	glucose	fructose	sucrose	starch	soluble sugars per starch	sucrose per starch
D	2.24 ± 0.4	1.27 ± 0.3	3.31 ± 0.5	4.25 ± 0.5	1.6	0.78
J2	3.36 ± 0.4	1.20 ± 0.3	2.39 ± 0.3	2.60 ± 0.3	2.7	0.92

[a] Leaves were harvested after 8 h of illumination; data were obtained from four independent transgenic lines (J2.51, J2.52, J2.53, and J2.54), and each line was represented by at least six plants.

the DNA G-box via a 67 kDa protein. The broad spectrum of activities that are affected by 14-3-3 proteins indicates their usefulness in plant metabolism modification. To prove this hypothesis, transgenic potato plants overexpressing the 14-3-3 protein were created and initially characterized (*8*).

Greenhouse-Grown Plant Tubers. 14-3-3 sense plants maintained in tissue culture were visually indistinguishable from nontransformed control plants. When grown in the greenhouse, again there was no dramatic change in the phenotype of the aerial parts of the transgenic plants. However, the plants overexpressing the 14-3-3 protein flowered earlier than the control plants, and the leaves of the transgenic potato plants contained more chlorophyll and lost it more slowly than the control plants (*8*). It should be noted that a significant change in the phenotype of the tubers formed was observed (Table 1).

The sense transgenic plants grown under standard greenhouse conditions showed an increase in tuber number and a decrease in tuber size. A significant decrease in the starch content of the transgenic tubers was detected.

Greenhouse-Grown Plant Leaves. There was significant alteration in the carbohydrate content of leaves from plants grown in the greenhouse with elevated levels of the 14-3-3 protein (Table 2).

The results presented in Table 2 show that increased levels of the 14-3-3 protein significantly affect the ratio of soluble sugars to starch in leaves and that this resulted from an increase in glucose content and a decrease in starch synthesis.

In tubers a decrease in the starch content of sense transgenic plants was also detected (Table 1), but the fresh weight of tuber per plant was only slightly higher than for the control.

Catecholamine Content in Potato Leaves. The striking change in carbohydrate content in transgenic J2 plants gives rise to the question of how the 14-3-3 protein is involved in carbohydrate metabolism. A possible mechanism through catecholamine regulation was considered. It is suggested that the 14-3-3 protein regulates tyrosine hydroxylase activity, the rate-limiting enzyme in the catecholamine synthesis pathway (*22*).

Table 3. Catecholamine Content in the Leaves of the Control (D) and Transgenic Potato Plants with Overexpression (J2) of the 14-3-3 Protein

	catecholamine content (nmol/g of FW)		
	dopamine	norepinephrine	methylnorepinephrine
D	45.4 ± 6.8	77.4 ± 8.7	2.7 ± 0.3
J2	99.3 ± 9.1	95.2 ± 9.8	4.4 ± 0.5

[a] Data were obtained from four independent transgenic lines selected from the J2 transgene with four to six plants representing each line.

It is known that catecholamines in animal cells regulate glycogen metabolism. Epinephrine and norepinephrine markedly stimulate glycogen breakdown, and their action is mediated by cyclic AMP. Due to this, this study took into account catecholamine content in the control and transgenic potato plants.

The HPLC examination of catecholamines in protein-free extracts of leaves from the control and transgenic plants was done with the use of a Bio-Rad kit commonly used in clinical laboratories for urine examination. On the basis of the retention time (RT), dopamine (RT = 9.66) and norepinephrine (RT = 3.85) can be identified. It should be pointed out that epinephrine (RT = 4.41) was not detected; methylnorepinephrine (RT = 4.72) was found instead. Dihydroxybenzylamine used as internal standard (IS) gave a retention time of ~6.20.

A very similar HPLC profile was obtained by Takimoto et al. (*23*) when an extract from *Lemna paucicostata* was analyzed. The authors reported an important effect of norepinephrine on the induction of plant flowering. It should be noted that transgenic potato plants overexpressing the 14-3-3 protein flower earlier than the control plants. The catecholamine content in the leaves of the control and transgenic plants measured with the HPLC method is presented in Table 3. Plants overexpressing the 14-3-3 protein produce more catecholamines than control plants.

The amount of catecholamines in the tubers is much lower. The values for norepinephrine in the control and transgenic J2 plants are 57.6 and 169.0 pmol/g of fresh weight (FW), respectively.

Carbohydrate Analysis of Transgenic Potato Tubers from the Field Trial. To prove the results obtained from transgenic plants grown in standard greenhouse conditions, the field trial experiment was performed, and the data are presented in Table 4.

In four years of experiments, only slight changes in several measured parameters were found. The increase in the 14-3-3 protein level does not clearly affect tuber fresh weight per plant and mean fresh weight per tuber. Thus, the field experiments do not confirm the results obtained for transgenic plants grown in greenhouse conditions.

Analysis of carbohydrate content in the tubers from the last three years of field trials is presented in Table 5. In the first two years of the experiment, the carbohydrate content in the transgenic tuber from the field trial altered in the same way as was observed for the greenhouse-grown plants. A decrease in starch level concomitant with an increase in glucose content was a characteristic feature of those plants. The transgenic tuber produced in the last year showed an increase in starch content, contrary to the greenhouse plants and those from 1998 and 1999, whereas the glucose content

Table 4. Field Trials (1997−2000) of Potato Wild Type (D) and Transgene with Overexpression (J2) of the 14-3-3 Protein[a]

	tuber FW (g) per plant				tuber no. per plant				mean FW (g) per tuber			
plant	1997	1998	1999	2000	1997	1998	1999	2000	1997	1998	1999	2000
D	539 ± 30	646 ± 35	420 ± 40	532 ± 35	10.1 ± 0.4	8.3 ± 0.2	7.0 ± 0.3	7.9 ± 0.4	53.2	78.0	60.0	67.0
J2	476 ± 25	623 ± 25	340 ± 25	618 ± 30	11.4 ± 0.3	7.8 ± 0.3	6.7 ± 0.2	7.3 ± 0.3	41.8	80.0	51.0	83.0

[a] Seventy-five plants of each type (control and transgene) were grown.

Table 5. Carbohydrate Content in the Tubers of Control (D) and Transgenic Potato Plants with Overexpression (J2) of the 14-3-3 Protein

	starch % DW			sucrose % DW			hexose % DW			soluble sugar/starch $\times 10^{-2}$		
plant	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
D	66.0 ± 6.4	69.2 ± 5.0	75.8 ± 4.5	2.5 ± 0.1	0.6 ± 0.08	1.6 ± 0.10	1.9 ± 0.1	0.6 ± 0.06	0.6 ± 0.05	6.7	1.7	2.9
J2	61.0 ± 7.0	68.4 ± 4.5	79.9 ± 6.4	1.4 ± 0.2	0.8 ± 0.04	1.1 ± 0.08	3.7 ± 0.4	0.8 ± 0.04	0.7 ± 0.02	8.4	2.3	2.3

[a] Seventy-five plants of each type (control and transgene) were grown.

Table 6. Protein, Vitamin C, and Lipid Contents in the Tubers of Wild Type (D) and Transgenic Potato Plants with Overexpression (J2) of the 14-3-3 Protein in Percent of Dry Weight

				lipids % DW		
plant	dry wt %	protein % DW	vitamin C mg% DW	total	nonpolar	polar
D	19.6 ± 1.4	12.1 ± 1.3	112.6 ± 4.9	0.52 ± 0.03	0.13 ± 0.01	0.32 ± 0.01
J2	18.7 ± 0.9	12.5 ± 0.9	125.3 ± 1.5	0.88 ± 0.02	0.35 ± 0.01	0.40 ± 0.03

in the tubers harvested last year was again increased as in all of the other transgenic plants. Thus, the characteristic feature of plant tubers with 14-3-3 protein overexpression verified in the field experiments is the increase in their glucose content.

Protein and Lipid Analysis of Transgenic Potato Tubers from the Field Trial. Because the 14-3-3 protein regulates in vitro nitrogen (nitrate reductase) and carbon (sucrose phosphate synthase) fixation, it was of interest to analyze the protein and lipid contents in transgenic potato tubers overexpressing the 14-3-3 protein. The results of analyses on one year's harvest are presented in Table 6.

Overexpression of the 14-3-3 protein does not affect protein synthesis and vitamin C content, but it does affect lipid content. The potato tuber is a rich source of vitamin C.

The fat (total lipids) in potato tubers is present in small amounts, and its content in various strains of potato is up to 0.5% of dry tuber mass (*24*). The total fat in potato tubers mainly consists of phospholipids (47%), glyco- and galactolipids (22%), both of which are structural elements of biological membranes, and neutral lipids such as acylglycerols and free fatty acids (21%). The composition of the fatty acids of fat isolated from tubers is especially nutritionally advantageous, because the essential part of all fatty acids is formed by unsaturated fatty acids with one to three double bonds, mainly linolenic acid (40−50%) (*25*, *26*). However, the small fat content in potato in general means that the daily intake of this valuable fat from potato is minimal; the amounts of linoleic and linolenic acids in 100 g of the edible parts of tubers are 32.13 and 22.75 mg, respectively (*27*). Because of this, growing species of potato that would store more fat in their tubers would be an important task.

The data presented in Table 5 showed that the control plant tuber contains 0.52% fat, and in tubers of transgen J2 a 69% increase in the total fat content was observed. The tubers of the control plants contained a small amount of nonpolar lipids (0.13%), whereas the transgen demonstrated an almost triple increase in this lipid fraction. In the control plants the polar lipids composed 0.32% of the dry mass, and the increase of this fraction in modified tubers was only slight (25%). A comparison of the percentage of polar and nonpolar fractions in the total fat from the investigated potato tubers revealed that significantly different ratios of these fractions were observed in J2 potato when compared to the wild-type plant. In the control tubers the neutral lipids composed 25.6% and the polar lipids 61.2% of the total fat. In J2 potato tubers the fraction of nonpolar lipids was >50% higher, whereas the polar lipid fraction was ∼20% lower than for the control. These results indicate that the increase in the fat content in modified potato was mainly caused by a synthesis of a greater amount of neutral lipids in tubers.

Fatty Acid Content in Potato Tubers from the Field Trial. The result of the fatty acid analysis of the fat extracted from tubers (Figure 1) from one year's harvest showed that the percentage of these acids is in great accordance with the published data (*25*, *26*). The content of linoleic acid, which is the main fatty acid of potato tubers, came to 49% of all acids. The fat from the control tubers additionally contained a large amount of palmitic and linolenic acids (10 and 14% of all fatty acids, respectively). Fatty acids found in the fat of transgenic J2 tubers were present in proportions similar to those in the control plants. The only exception was a 55% increase in oleic acid, but the content of this acid is only 4.5% of all fatty acids in the J2 potato.

The percentage composition of fatty acids in the nonpolar fraction of tuber lipids is presented in Figure 2. It was found that the main fatty acids of this lipid fraction were palmitic, linoleic, and linolenic acids. In the case of the control plants the contents of these acids were 40, 21, and 13% of the sum of all fatty acids, respectively. In the transgenic potato a significant elevation of the unsaturated fatty acid component of the nonpolar fraction of tuber fat was revealed. The linoleic acid content increased by 48% and the linolenic acid by 33%. A significant 71% increase of oleic acid was also observed, whereas the palmitic acid content decreased by ∼43%.

The percentage contribution of the polar fraction to the total fat of tubers was more than twice the contribu-

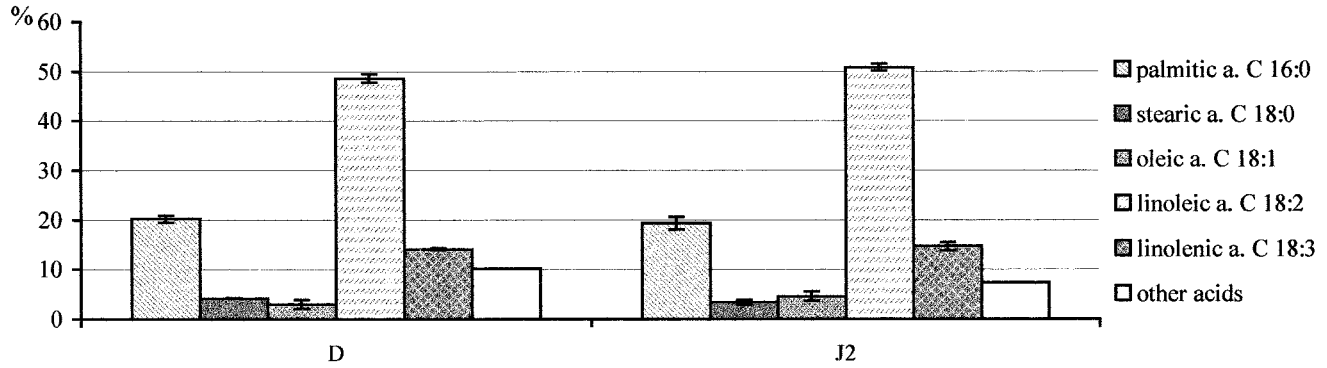

Figure 1. Relative composition of the fatty acids in the tubers of wild-type (D) and transgenic potato plants with overexpression (J2) of 14-3-3 protein.

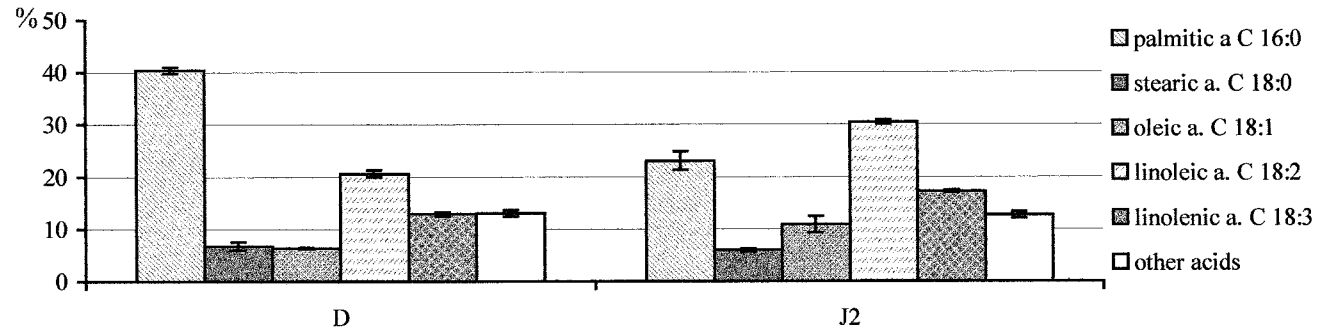

Figure 2. Relative composition of fatty acids in the nonpolar fraction of tuber lipids from wild-type (D) and transgenic potato plants with overexpression (J2) of 14-3-3 protein.

tion of the nonpolar fraction. Moreover, in the nonpolar lipids the participation of palmitic acid was observed to be twice as high as in the total fat. In the case of the transgenic J2 tubers the increase of the nonpolar fraction was accompanied by a significant increase in the unsaturated fatty acids in this fraction.

Conclusions. The manipulation of 14-3-3 protein contents in potato plants grown in the greenhouse resulted in significant changes in tuber size and yield. Clearly, overexpression of the 14-3-3 protein in potato plants resulted in a decrease in tuber size and starch content. However, the effect was less clear when transgenic tubers from the field trial were analyzed. The feature that consistently differentiated the transgenic tubers from plants grown in greenhouse conditions and from the field test was their increase in glucose content. The mechanism by which the 14-3-3 protein regulates carbohydrate metabolism in potato plants possibly involves the regulation of catecholamine synthesis, presumably by affecting tyrosine hydroxylase activity. As in animal cells, catecholamines may activate cAMP synthesis, which in turn activates starch mobilization.

In the examined transgenic potato tubers, overexpression of the 14-3-3 protein resulted in a significant increase in total fat content. It should be pointed out that within total fat, a significant increase of the nonpolar lipid fraction in the modified tubers was noted.

The fat from both modified and control potato tubers revealed a nutritionally valuable composition of fatty acids, with a high content of unsaturated acids.

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