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BIOCHEMICAL COMPOSITION OF SEEDS OF TRANSGENIC SPRING RAPESEED PLANTS CARRYING THE MAMMALIA *CYP11A1* GENE

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The qualitative and quantitative fatty-acid seed composition was identified by the gas chromatography method, and the Kjeldahl method was used to identify the total protein in the fresh mass in T_0—T_3 generations of transgenic spring rapeseed lines of Magnat variety, the Belarusian breeding. Transgenic lines were previously developed by *Agrobacterium*-mediated transformation using a construct carrying transcriptionally active heterologous genes: mammalian — *cyp11a1* of cytochrome P450scc and bacterial — *bar*. Biometric analysis allowed to establish a stable increase in the mass of 1000 seeds and main raceme indices (length, the number of pods and side shoots) in these lines. Biochemical analysis showed that the insertion of the heterologous *cyp11a1* gene does not affect the qualitative seed oil composition of transgenic lines, while significant changes were noted in the quantitative ratio of fatty acids in the seeds of transgenic lines in T_0—T_3 generations as compared to the control plants.

Key words: *Brassica napus* L. var. *oleifera* DC., *cyp11a1* gene, cytochrome P450scc, seeds, fatty acids, total protein.

Today, rapeseed is one of the main in-demand cultures on the global market. The seeds of rape contain up to 49 % of oil. Rapeseed oil has an increased biological value and is equal to olive oil in terms of its fatty-acid content. Oil cake and grist obtained after oil extraction are used as protein-rich (up to 33 %) animal feed [1, 2].

Genetic engineering methods allow to develop rapeseed plants with new valuable traits. In order to develop transgenic rapeseed plants with new valuable traits, scientists use the heterologous genes of different origin. In our study, we used the Mammalia gene, namely, the *cyp11A1* gene from the bovine adrenal cortex, which encodes the mitochondrial cytochrome P450scc. Cytochrome P450scc is a 20,22-desmolase, which forms pregnenalone, a precursor of all types of steroid hormones, during the reaction of cholesterol hydroxylation at C22, C20 positions with subsequent splitting

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of the side chain [3, 4]. Cytochrome P450scc is synthesized in the cytoplasm in the form of a precursor protein carrying an N-terminal target sequence. Cytochrome P450scc undergoes final proteolytic processing in mitochondria and is incorporated into their inner membrane [5, 6]. It was believed that the target sequence specificity determines the tissue specificity of the precursor protein P450scc [7]. However, the precursor protein import and the P450scc expression in plant mitochondria, as well as the functional activity of native P450scc *in vivo,* were shown *in vitro* [8, 9].

Previously it was also thought that the biological role of *cyp11A1* is limited only to the conversion of cholesterol into pregenenalone, but *in vitro* it was demonstrated that as a substrate purified bovine cytochrome P450scc may use not only cholesterol, but also other sterols and not only of animal origin [10]. The main stages of metabolism of animal steroid hormones and plant brassinosteroids are similar [3, 11—13]. The formation of progesterone during the biosynthesis of cardiac glycosides in foxglove plants and its influence on the regulation of plant growth and development were shown [14—16]. Under the influence of exogenous progesterone, flowering is induced in *Arabidopsis*, and in tobacco, the growth of pollen tubes in *in vitro* culture is stimulated [17]. In experiments on the tobacco plant transformation, the effect of the *cyp11A1* gene on the growth, development, and physiological and biochemical characteristics of plants was demonstrated [18]. Therefore, it is possible to assume that brassinosteroids biotransformation processes mediated by cytochrome P450scc will be occurring in plants.

The aim of our study was to investigate the effect of the *cyp11A1* gene of animal origin in genome of transgenic spring rapeseed plants on rapeseed biochemical traits, such as the fatty-acid seeds composition and the total protein in the fresh mass, compared to control plants over a number of generations.

Materials and methods

Plant material. The object of study — 8 transgenic lines of spring rape in T_0—T_3 generations (*Brassica napus* L. var. *oleifera* DC.), developed on the basis of Magnat variety of the Belarusian selection, grown under controlled environment. Such transgenic lines were obtained as a result of *Agrobacterium*-mediated transformation and carry a transcriptionally active insertion of heterologous genes of animal (*cyp11A1*) and bacterial (*bar*) origin in their genome [19, 20]. Biometric and correlation analysis showed the effect of the *cyp11A1* gene on the genome of transgenic rapeseed plants resistant to Basta herbicide in their T_1—T_3 generations [21].

For a more detailed study of the influence of the *cyp11A1* gene on the plant traits, we determined the qualitative and quantitative composition of fatty acids in seeds and the total amount of protein in the fresh mass in T_0—T_3 generations of 8 transgenic lines and control plants.

Determining fatty-acid composition in seeds. Biochemical analysis of the fatty-acid composition of oil in the seeds of transgenic lines and the control plants were performed on the gas chromatograph «Chromatec-Crystall 5000» with the automatic liquid dispenser ALD-2M on the capillary column SolGelWax 30 m × 0.25 mm × 0.5 μm in a carrier gas current — heli-

um at a speed of 25 cm/s with temperature programming in the range of 185–240 °C. Methyl esters were obtained and chromatographed in accordance with GOST R 51486-99 «Vegetable oils and animal fats. Obtaining fatty acid methyl esters» and GOST R 51483-99 «Vegetable oils and animal fats. Determining by gas chromatography the mass fraction of methyl esters of individual fatty acids to their sum».

Determining total protein in a fresh mass. Determining total protein in a fresh mass of transgenic and control plants of spring rapeseed was carried out using GOST ISO 5983-97 «Feed, animal feed, feed raw material. Determining the nitrogen mass fraction and estimating the proportion of the crude protein fraction. The Kjeldahl method». The essence of this method is in ashing of the organic matter of the analyzed sample with sulfuric acid in the presence of a catalyst, in alkalizing of the reaction product, distilling and titrating of released ammonia, estimating the nitrogen mass fraction and the crude protein mass fraction by multiplying the result obtained by the conversion factor of the nitrogen mass fraction by 6.25 per crude protein mass fraction.

Statistical analysis. Data is presented in the form of an average value and a triplicate mean error. In order to determine the significance of differences, the Student's *t*-test was applied at $p = 0.05$. The relationship between the quantitative ratio of unsaturated fatty acids was assessed using correlation analysis.

Results and discussion

Earlier, using the statistical processing of biometric experimental data, we established the effect of cDNA of the *cyp11A1* gene of bovine mitochondrial cytochrome P450scc on the recipient genome of spring rapeseed, namely a stable increase in the mass of 1000 seeds in T_1–T_3 generations and the main raceme indices: length, the number of pods and lateral shoots [21]. Out of 8 analyzed transgenic rapeseed lines in a number of generations, Bn9/93/3 and Bn9/93/2 lines in the T_1 generation had the lowest morphometric parameters and in the T_2 generation turned out to be sterile, which is explained by the phenomenon of heterostyly [22].

Using the chromatographic method, the oil composition was determined by 13 fatty acids in the seeds of transgenic and control lines (Tables 1–4). The results of a four-year qualitative analysis of fatty acids in transgenic lines showed that the pool of determined fatty acids consists of omega-3, omega-6 and omega-9 fatty acids and corresponds to those in the control samples. The quantitative ratio analysis showed that monounsaturated fatty acids constituted the main part of 13 determined profiles, and among them oleic acid accounted for the maximum content — from 63.3 % (Bn9/93/19 line, T_3 generation) to 77.46 % (Bn9/93/13 line, T_3 generation). For the control, this index was in the range of 63.13–64.39 %.

In the T_0 generation, the transgenic line Bn9/93/3 had the maximum oleic acid content (73.49 %), which was by 9.9 % higher as compared to the control, and the minimum was for Bn9/93/14 (70.48 %) (Table 1). Although line Bn9/93/14 had a reduced oleic acid content among the samples of transgenic lines, it still exceeded the control by 6.89 %. On average

TABLE 1. Fatty-acid composition of the seed oil of spring rapeseed transgenic lines in the T_0 generation, % of the acids sum

Fatty acid	Line								
	Bn9/93/2	Bn9/93/3	Bn9/93/6	Bn9/93/9	Bn9/93/13	Bn9/93/14	Bn9/93/19	Bn9/93/21	Control
Palmitic (16:0)	3.93	3.85	3.92	3.99	3.91	4.03	3.82	3.93	3.98
Palmitoleic (16:1)	0.15	0.16	0.17	0.16		0.17	0.16	0.16	0.17
Stearic (18:0)	1.64	2.04	1.78	1.51	0.63	2.24	1.87	1.73	1.93
Oleic (18:1)	71.72	73.49	71.80	71.82	71.64	70.48	71.12	71.26	63.59
Linoleic (18:2)	13.56	11.92	13.04	14.96	13.52	13.90	14.08	14.43	17.72
Linolenic (18:3)	6.23	5.25	5.63	4.75	6.36	5.42	5.77	5.42	7.93
Arachidic (20:0)	0.62	0.80	0.73	0.64	0.62	0.82	0.70	0.70	0.63
Eicosenic (20:1)	1.37	1.47	1.72	1.30	1.35	1.67	1.43	1.45	2.34
Eicosadienoic (20:2)	0.06	0.05	0.06	0.05	0.05	0.06	0.05	0.06	0.08
Behenic (22:0)	0.36	0.74	0.43	0.38	0.37	0.52	0.51	0.43	0.33
Erucic (22:1)	0.03	0.04	0.27	0.02	0.08	0.23	0.06	0.09	1.01
Lignoceric (24:0)	0.17	0.30	0.26	0.26	0.15	0.28	0.23	0.20	0.13
Selacholic (24:1)	0.15	0.16	0.17	0.17	0.16	0.18	0.19	0.17	0.15
Σ fatty acids, %	100	100	100	100	100	100	100	100	100
Σ saturated fatty acids	6.72	7.73	7.12	6.54	5.68	7.89	7.13	6.99	7.00
Σ monounsaturated fatty acids	73.42	75.16	74.13	73.47	73.39	72.73	72.72	73.13	67.26
Σ polyunsaturated fatty acids	19.85	17.22	18.73	19.76	19.93	19.38	19.90	19.91	25.73

Σ fatty acids — the sum of fatty acids, Σ saturated fatty acids — the sum of saturated fatty acids, Σ monounsaturated fatty acids — the sum of monounsaturated fatty acids, Σ polyunsaturated fatty acids — the sum of polyunsaturated fatty acids.

in the experimental samples of transgenic lines in the T_0 generation, the oleic acid content was 71.67±0.30 %, which was 8.08 % higher than the content of oleic acid in the control sample.

In addition, one of the main indices of the nutritional value of rapeseed oil is the content of erucic acid, which should not exceed 2 %. In our samples, the erucic acid content in T_0—T_3 generations was 0.02—1.11 % for the Bn9/93/9 line of the T_0 generation and the Bn9/93/19 line of the T_3 generation respectively. Such oleic and erucic acids content corresponds to rapeseed oil with a high oleic acid content and a low erucic acid content. In nowaday oilseed rape breeding, varieties with such content of erucic acid (less than 2 %) and not more than 18—20 μmol/g of glucosinolate in seeds are considered double zero, and the products obtained from the processing of such varieties are safe for human and animal consumption [23].

TABLE 2. Fatty-acid composition of the seed oil of spring rapeseed transgenic lines in the T_1 generation, % of the acids sum

Fatty acid	Line						
	Bn9/93/6 (n=37)	Bn9/93/9 (n=50)	Bn9/93/13 (n=40)	Bn9/93/14 (n=30)	Bn9/93/19 (n=38)	Bn9/93/21 (n=30)	Control
Palmitic (16:0)	3.89	3.84	3.79	3.99	3.88	3.79	4.09
Palmitoleic (16:1)	0.14	0.16	0.13	0.15	0.17	0.16	0.18
Stearic (18:0)	1.79	1.57	2.10	1.86	1.94	1.62	1.96
Oleic (18:1)	72.86	70.12	75.29	73.92	70.76	72.38	64.39
Linoleic (18:2)	12.36	14.38	10.51	11.49	14.51	13.17	17.55
Linolenic (18:3)	5.31	6.56	4.32	4.83	5.61	5.57	7.69
Arachidic (20:0)	0.78	0.67	0.94	0.85	0.73	0.71	0.65
Eicosenic (20:1)	1.63	1.62	1.60	1.65	1.43	1.57	2.05
Eicosadienoic (20:2)	0.06	0.07	0.05	0.06	0.06	0.06	0.07
Behenic (22:0)	0.56	0.50	0.64	0.62	0.45	0.51	0.35
Erucic (22:1)	0.03	0.05	0.04	0.05	0.09	0.03	0.73
Lignoceric (24:0)	0.32	0.26	0.39	0.34	0.20	0.25	0.14
Selacholic (24:1)	0.26	0.20	0.21	0.20	0.17	0.18	0.15
Σ fatty acids, %	100	100	100	100	100	100	100
Σ saturated fatty acids	7.34	6.84	7.86	7.66	7.2	6.88	7.19
Σ monounsaturated fatty acids	74.92	72.15	77.27	75.97	72.62	74.32	67.50
Σ polyunsaturated fatty acids	17.73	21.01	14.88	16.38	20.18	18.80	25.31

Σ fatty acids — the sum of fatty acids, Σ saturated fatty acids — the sum of saturated fatty acids, Σ monounsaturated fatty acids — the sum of monounsaturated fatty acids, Σ polyunsaturated fatty acids — the sum of polyunsaturated fatty acids, n — number of plants.

With regard to the oleic acid content in the T_1 generation, transgenic lines exceeded the control by an average of 8.16 % and in some lines from 5.73 % (Bn9/93/9) to 10.8 % (Bn9/93/13) (Table 2). The maximum amount of oleic acid was contained in the seeds of the Bn9/93/13 line — 75.29 %, and the minimum — in the seeds of the Bn9/93/9 line (70.12 %). The erucic acid content in the experimental samples does not exceed 0.1 %.

The determination of fatty-acid oil composition in the seeds of transgenic lines of T_2 and T_3 generations showed that in the T_2 generation the line Bn9/93/9 (77.32 %) had the maximum oleic acid content, and the minimum of 70.50 % was in the line Bn9/93/13. The oleic acid content in the control seeds was 63.13 %, which is, on average, 10.64 % lower as compared to transgenic lines. In the T_3 generation, the maximum value of oleic acid corresponded to the line Bn9/93/13 — 77.46 %, which exceeds the control by 14.29 %, and the minimum — 63.13 % (Bn9/93/19). This index for the control was 63.17 % (Tables 3, 4).

A change of the oleic acid content in the seeds of transgenic lines had a positive trend in generations, namely in the T_0 generation —

TABLE 3. Fatty-acid composition of the seed oil of spring rapeseed transgenic lines in the T_2 generation, % of the acids sum

Fatty acid	Line						
	Bn9/93/6 (n=29)	Bn9/93/9 (n=30)	Bn9/93/13 (n=30)	Bn9/93/14 (n=30)	Bn9/93/19 (n=30)	Bn9/93/21 (n=30)	Control
Palmitic (16:0)	3.87	3.88	3.60	3.84	3.87	3.83	4.12
Palmitoleic (16:1)	0.14	0.13	0.15	0.13	0.14	0.17	0.18
Stearic (18:0)	2.00	2.30	1.39	1.89	2.33	1.73	1.97
Oleic (18:1)	72.58	77.32	70.50	74.19	76.35	71.70	63.13
Linoleic (18:2)	12.37	8.64	14.48	11.40	9.43	13.86	17.95
Linolenic (18:3)	5.36	3.52	6.79	4.67	3.75	5.68	7.67
Arachidic (20:0)	0.85	1.02	0.061	0.85	1.01	0.71	0.66
Eicosenic (20:1)	1.61	1.66	1.60	1.69	1.63	1.42	2.41
Eicosadienoic (20:2)	0.08	0.05	0.06	0.06	0.05	0.06	0.08
Behenic (22:0)	0.59	0.73	0.41	0.63	0.70	0.45	0.38
Erucic (22:1)	0.04	0.04	0.04	0.05	0.07	0.03	1.11
Lignoceric (24:0)	0.33	0.48	0.19	0.37	0.45	0.22	0.16
Selacholic (24:1)	0.19	0.23	0.18	0.22	0.22	0.15	0.18
Σ fatty acids, %	100	100	100	100	100	100	100
Σ saturated fatty acids	7.64	8.41	6.20	7.58	8.36	6.94	7.29
Σ monounsaturated fatty acids	74.56	79.38	72.47	76.28	78.41	73.47	67.01
Σ polyunsaturated fatty acids	17.81	12.21	21.33	16.13	13.23	19.60	25.70

Σ fatty acids — the sum of fatty acids, Σ saturated fatty acids — the sum of saturated fatty acids, Σ monounsaturated fatty acids — the sum of monounsaturated fatty acids, Σ polyunsaturated fatty acids — the sum of polyunsaturated fatty acids, n — number of plants.

71.67±0.3 %, in the T_1 generation — 72.55±0.78 %, in the T_2 generation — 73.77±1.09 %, and in the T_3 generation — 72.56±2.11 %, which is 8.17 %, 8.16 %, 10.64 % and 9.39 % higher than the control respectively. Rapeseed oil with high oleic acid content has a low oxidizing capacity, pleasant smell and taste; it has a better shelf life and is beneficial to human health. All of the above qualities significantly increase the consumer characteristics of rapeseed oil and make it suitable for use in the food industry [24].

Nutritional value of rapeseed oil is determined not only by the quantitative content of oleic acid, but also by the ratio of polyunsaturated (linoleic, linolenic) and monounsaturated (oleic) fatty acids, being one of the most important goals of rapeseed breeding at the global level [23]. In our study, the highest level of linolenic acid (18:3) was found in the Bn9/93/19 line of the T_3 generation (7.67 %), while its lowest amount was found in the Bn9/93/13 line of the T_3 generation (3.43 %). In the control samples, the content of linolenic acid varied from 7.64 to 7.93 %. Linoleic acid content in seeds of transgenic lines ranged from 8.62 % (Bn9/93/13 line,

TABLE 4. Fatty-acid composition of seed oil of spring rapeseed transgenic lines in the T_3 generation, % of the acids sum

Fatty acid	Line						
	Bn9/93/6	Bn9/93/9	Bn9/93/13	Bn9/93/14	Bn9/93/19	Bn9/93/21	Control
Palmitic (16:0)	3.90	3.74	3.90	3.93	4.12	3.73	4.01
Palmitoleic (16:1)	0.18	0.12	0.12	0.12	0.18	0.13	0.18
Stearic (18:0)	1.78	1.73	2.29	1.76	1.97	1.82	1.97
Oleic (18:1)	70.39	73.85	77.46	75.74	63.13	74.77	63.17
Linoleic (18:2)	13.71	12.11	8.62	10.23	17.95	11.53	17.60
Linolenic (18:3)	6.79	5.13	3.43	4.21	7.67	4.66	7.64
Arachidic (20:0)	0.73	0.74	1.03	0.85	0.66	0.77	0.63
Eicosenic (20:1)	1.56	1.51	1.67	1.74	2.41	1.54	2.66
Eicosadienoic (20:2)	0.06	0.06	0.05	0.06	0.08	0.06	0.08
Behenic (22:0)	0.50	0.57	0.73	0.69	0.38	0.53	0.37
Erucic (22:1)	0.05	0.07	0.05	0.07	1.11	0.05	1.37
Lignoceric (24:0)	0.19	0.20	0.45	0.37	0.16	0.25	0.14
Selacholic (24:1)	0.17	0.17	0.20	0.21	0.18	0.17	0.16
Σ fatty acids, %	100	100	100	100	100	100	100
Σ saturated fatty acids	7.10	6.98	8.40	7.60	7.29	7.10	7.12
Σ monounsaturated fatty acids	72.35	75.72	79.50	77.88	67.01	76.66	67.54
Σ polyunsaturated fatty acids	20.56	17.30	12.10	14.50	25.70	16.25	25.32

Σ fatty acids — the sum of fatty acids, Σ saturated fatty acids — the sum of saturated fatty acids, Σ monounsaturated fatty acids — the sum of monounsaturated fatty acids, Σ polyunsaturated fatty acids — the sum of polyunsaturated fatty acids, n — number of plants.

T_3 generation generation) to 17.95 % (Bn9/93/19 line, T_3 generation). Linoleic acid indices for the control fluctuated in the range of 17.55—17.95 %.

The content of saturated fatty acids, palmitic and stearic, in the seeds of transgenic lines T_0—T_3 generations varied from 3.60—4.12 and 1.39—2.30 % respectively. For the control, the indices were 3.98—4.12 % and 1.93—1.97 % respectively.

Based on the results obtained, it can be concluded that the main fatty acids that make up the seed oil of transgenic lines are palmitic, oleic, linoleic and linolenic. The content of other fatty acids, such as stearic and eicosenoic, in the seed oil of the experimental samples was not high and, on average, did not exceed the control, while the content of such acids as behenic, arachidic and lignoceric did not significantly increase in transgenic lines.

When assessing the quantitative ratio of unsaturated fatty acids in a number of generations, an inverse correlation was established between the content of oleic and linoleic acids and oleic and linolenic acids; a positive correlation was found between linoleic and linolenic acids (at $p = 0.05$). The correlation coefficient between the content of oleic and linoleic acids

TABLE 5. Total protein content in the fresh mass of rapeseed lines in T_0—T_3 generations, % per absolutely dry matter

Line	Generation				
	T_0	T_1	T_2	T_3	T_0—T_3
Control	31.85±0.14 (n=1)	31.61±0.64 (n=25)	31.50±0.25 (n=18)	30.74±0.55 (n=18)	31.34±0.20 (n=62)
Bn9/93/2	31.77±0.30 (n=1)	31.07±0.56 (n=25)	–	–	31.41±0.32 (n=26)
Bn9/93/3	30.67±0.30 (n=1)	27.37±1.32 (n=64)	–	–	29.02±1.56 (n=65)
Bn9/93/6	29.87±0.18 (n=1)	25.09±0.54 (n=37)	30.48±4.09 (n=29)	26.78±0.48 (n=13)	28.06±1.11 (n=80)
Bn 9/93/9	31.11±0.44 (n=1)	34.25±4.13 (n=50)	30.42±1.75 (n=30)	32.92±1.15 (n=14)	32.17±1.09 (n=95)
Bn9/93/13	29.80±0.24 (n=1)	26.19±0.19 (n=40)	24.44±0.84 (n=30)	26.46±2.11 (n=12)	26.72±0.76 (n=83)
Bn9/93/14	30.57±0.29 (n=1)	32.26±0.48 (n=30)	29.12±0.27 (n=30)	27.83±1.85 (n=11)	29.94±0.65 (n=72)
Bn9/93/19	30.07±0.06 (n=1)	28.19±5.09 (n=38)	25.80±0.61 (n=30)	23.00±1.83 (n=12)	26.76±1.41 (n=81)
Bn9/93/21	30.11±0.09 (n=1)	35.94±2.16 (n=30)	29.51±1.29 (n=30)	30.94±0.56 (n=11)	31.63±0.94 (n=72)

n — number of plants.

in the samples of transgenic plants was in range $-0.99 < r > -0.95$, and between oleic and linolenic acids $-0.99 < r > -0.89$. A close direct relationship was observed between linoleic and linolenic acids — the correlation coefficient was in range $0.74 < r > 0.99$. For the control, these indices were as follows: between oleic and linoleic acids $r = -0.62$ and between oleic and linolenic acids $r = -0.14$; no close correlation ($r = 0.05$) was found between linoleic and linolenic acids in the control sample.

The inverse correlation between monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic and linolenic acids) may be explained by the influence of agroclimatic conditions. An oil unsaturation degree usually increases along with a temperature decrease during the period of an intensive oil-forming process. Oleic acid content in plants is usually increased in the areas with warm and humid climat [25—29]. Moreover, the synthesis of saturated fatty acids in the seeds of oil plants occurs from the start of maturation, and at the final stages of maturation, an active synthesis of unsaturated fatty acids is initiated.

In our case, differences by the content of individual fatty acids and their ratio in the oil of transgenic lines and the control may be determined not only by the agroclimatic conditions of growing, but genotype specifics as well, namely the effect of the *cyp11A1* gene of animal origin on the plant genome.

One of the most important indices of product quality, which determines its nutritional value, is the protein content. Rapeseed is the second cultivated oilseed crop in the world after soybean yielding a high-protein meal, which is used for feeding of cattle, poultry, pigs and fish [1, 2].

In our case, the Kjeldahl protein determination method, which is generally recognized for the analysis of raw material and finished products, was used for identifying crude protein (Table 5).

The Bn9/93/21 line had the maximum protein content value — 35.94±2.16 % per absolutely dry matter over the four-year study period. When comparing the protein content in two samples, it is seen that in generations individual transgenic lines exceed the control. A four-year analysis of the average data obtained showed that only two transgenic lines exceeded the control — Bn9/93/21 (31.63±0.94) and Bn9/93/9 (32.17±1.09). Differences by the protein content in the control and transgenic lines may depend on the transgene expression level and plant genome insertion site.

From the transgenic lines analyzed, three promising lines characterized by the maximum content of oleic acid in different generations, namely Bn9/93/3 of the T_0 generation (73.49 %), Bn9/93/13 of the T_3 generation (77.46 %) and Bn9/93/19 of the T_2 generation (76.35 %), were identified.

Thus, on the basis of the data obtained, it can be concluded that the heterologous *cyp11A1* gene of animal origin from the bovine adrenal cortex, encoding the mitochondrial cytochrome P450scc, affects the quantitative composition of fatty acids and their ratio in the oil of transgenic spring rape seeds, and the total protein content in a fresh mass.

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БІОХІМІЧНИЙ СКЛАД НАСІННЯ ТРАНСГЕННИХ РОСЛИН ЯРОГО РІПАКУ, ЩО НЕСУТЬ ГЕН MAMMALIA *CYP11A1*

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Визначено якісний і кількісний жирнокислотний склад насіння методом газової хроматографії і загальний білок у свіжому матеріалі методом Кьєльдаля в T_0—T_3 поколіннях трансгенних ліній ярого ріпаку сорту Магнат білоруської селекції. Трансгенні лінії були раніше створені методом *Agrobacterium*-опосередкованої трансформації за допомогою конструкції, що несе транскрипційно активні гетерологічні гени: ссавців — *cyp11a1* цитохрому P450scc і бактеріальний — *bar*. За допомогою біометричного аналізу в даних лініях встановлено стабільне збільшення маси 1000 насінин та показників головної китиці (довжини, кількості стручків і бічних пагонів). В результаті біохімічного аналізу було показано, що інсерція гетерологічного гена *cyp11a1* не впливає на якісний склад олії насіння трансгенних ліній, однак відзначено істотні зміни в кількісному співвідношенні жирних кислот в насінні трансгенних ліній в T_0—T_3 поколіннях порівняно з контрольними рослинами.

Ключові слова: *Brassica napus* L. var. *oleifera* DC., ген *cyp11a1*, цитохром P450scc, насіння, жирні кислоти, загальний білок.