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PLANT GENETICS

The Use of Specific DNA Markers for the Identification of Alleles of the *FAD3* Genes in Rape (*Brassica napus* L.)

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Abstract—A search was conducted for the alleles responsible for the quality of food-grade rapeseed oil in a collection of 21 samples of spring and winter oilseed rape of Belarusian and Russian breeding. We also developed A- and C-gene-specific DNA markers to assess the genomic polymorphisms of rape for *FAD3* genes and selected plants with a low content of linolenic acid for use in the selection process. The development of a method for identifying *FAD3* alleles, which control the level of linolenic acid in rapeseed oil, as well as of the design for new dCAPS markers, enabled the identification of plants homozygous for individual *FAD3A* and/or *FAD3C* genes in the F_2-generation. These plants are currently involved in the selection process of new varieties with a reduced content of linolenic acid in rapeseed oil.

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INTRODUCTION

The quality of rapeseed oil is determined by the content of constituent fatty acids [1], which are represented by high-molecular unsaturated acids: oleic (C18:1), linoleic (C18:2), and linolenic (C18:3). Standard rapeseed oil is 50–60% oleic acid, 18–22% linoleic acid and 12–15% linolenic acid [1, 2]. One of the main directions in the selection of food grade oilseed rape is a reduction of the linolenic acid content to 2–4%, an increase in the oleic acid content to 80%, a reduction in the oil oxidation rate, and improvement in the quality of its composition.

High and low oleic forms of the rape were produced by mutagenesis [3, 4]. The content of oleic acid is controlled by the fatty acid desaturase 2 (*FAD2*) gene, which encodes the enzyme endoplasmic delta 12-oleate desaturase. This enzyme is responsible for the dehydrogenation of oleic acid to linoleic [5, 6]. Scheffler et al. [7] mapped four gene loci of *FAD2* on four different linkage groups, two of which belong to the A genome (*Brassica rapa*) and two of which belong to the C genome (*Brassica oleracea*). It has been established that the functional copies of the *FAD2* gene belong to the N5 linkage group of the A genome [7, 8].

Genetic analysis of the mapped population from a cross of a mutant with a linolenic acid level of 3% and the Drakkar variety (9–10%) showed that low levels of linolenic acid are controlled by the two major QTL-loci—L1 and L2—with an additive effect [9]. The two loci controlling the content of linolenic acid correspond to the two *FAD3* genes (fatty acid desaturase 3) encoding endoplasmic delta 15-linoleate desaturase, which is responsible for the dehydrogenation of linoleic acid to linolenic. One of the genes is localized in the A genome (*B. rapa*), and the other gene localized in the C genome (*B. oleracea*) of rape [9, 11]. Yang et a1. [10] found that single nucleotide substitutions in *BnaA.FAD3b* and *BnaC.FAD3b* can also lead to the realization of a phenotype with a low content of linolenic acid.

It is known that a single nucleotide mutation (C-to-T substitution) in the *FAD2* gene of the A genome leads to increased levels of oleic acid. As a result of the mutation, a stop codon (TAG) is formed, causing premature termination of the synthesis of the peptide chain. It was assumed that such a truncated polypeptide does not function, and that dehydrogenation of oleic acid does not occur, resulting in the accumulation of oleic acid in the mutant lines. In the studies that Hu et al. [2] and Micolaiczyk et al. [12] conducted on different mutant lines of winter and spring rape, single nucleotide mutations were found: C-to-T transitions in the seventh exon of the mutant allele of the *FAD3A* gene, the mutation of which leads to the substitution of arginine to cysteine, and G-to-A transitions in the 5'-donor splicing site of the sixth intron of the mutant allele of the *FAD3C* gene [2, 12]. A significant correlation was established between mutations in the A and C genomes and decreased levels of linolenic acid in the rapeseed oil [12].

One of the goals of modern rape breeding is the identification of genotypes with a high content of oleic and a low content of linolenic unsaturated fatty acids in the rapeseed oil. The production of molecular

Table 1. Allele-specific primers used to identify alleles of *FAD3A* and *FAD3C* genes

Primer	Sequence (5'–3')	The length of the amplified fragment, bp
LinAR	AATCTCTATCAATAGTTGTTAATCCTCCAG	339
LinAF	CTCGTTGGTCCAGTCACAGTTCTAA	
LinCR	CGTGAGTTCCAATATCGTGATGA	210
LinCF	ATGGTCACGATGATAAGCTGCCTTGGTACAGAGGCCAG	

markers for genes controlling traits with a complex nature of inheritance, such as the content of unsaturated fatty acids, enhance the informative content of data evaluation of these traits. It will enable a higher selection efficiency based on alleles associated with economically valuable traits in a large breeding population, which highly reduce the time for the production of newly selected valuable varieties and cultivars.

The goal of this study was to develop effective A and C specific genomic DNA markers in order to assess the genomic polymorphism in the rape genes determining the quality of food-grade rapeseed oil, to detect the alleles that determine a high content of oleic and a low content of linolenic unsaturated fatty acids in the collection of varieties, accessions, and variety-line hybrids of spring and winter rape of Belarusian and Russian selection, and to select forms with a low content of linolenic acid and involve them in the selection process.

MATERIALS AND METHODS

A collection of 21 samples of varieties of spring and winter rape of Belarusian and Russian breeding, variety-line hybrids, and an accession of Belarusian selection were used as materials for the study (Table 2).

For the selection of breeding material based on *FAD2* and *FAD3C* alleles that determine increased levels of oleic acid and decreased levels of linolenic acid in the oil, DNA markers according to [2] were used. PCR was done in a 25 μL reaction mixture containing a PCR buffer (650 mM Tris-HC1, 166 mM $(NH_4)_2SO_4$, 0.2% Tween 20, pH 8.8), 0.25 μM of each primer, 1 μM dNTP, 2.5 (1.5) mM $MgCl_2$, 1 U *Taq*-polymerase and 100 ng of total DNA. The amplification parameters for the detection of the mutant allele of *FAD2* were denaturation at 94°C for 4 min; 30 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s; and final elongation at 72°C for 30 min. The parameters for the detection of the *FAD3C* mutant allele were denaturation at 94°C for 4 min; 14 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s (the temperature for primer annealing was decreased by 0.7°C in each cycle); 20 cycles at 95°C for 30 s, 52°C for 30 s, 72°C for 30 s; and final elongation at 72°C for 7 min. The products of the amplification reaction were separated by electrophoresis in 0.8% agarose gel.

The two-stage amplification reaction method with the specific Fad3AR and Fad3CF primer pairs that we proposed earlier was used to differentiate alleles of *FAD3A* and *FAD3C* [12]. We also included specifically designed LinAF and LinCR primers [13] for genomic DNA fragments (A and C genome of rape) in the first amplification stage. In the second stage of detecting *FAD3* alleles, we used dCAPS primers that we designed: LinAF and LinAR for the A rape genome and LinCF and LinCR for the C rape genome [13]. The primer sequences are shown in Table 1. dCAPS primers were designed based on *FAD3* sequences of A and C genomes of *Brassica napus* L., which are represented in the GenBank database as EF488043.1 and EF488044.1. The composition of the PCR mixture was similar to the PCR mixture for the detection of *FAD2* and *FAD3C*. In the first stage, 10 μL of total DNA (100 ng) was used as the matrix; for the second stage of amplification, 2 μL of PCR product obtained during the first stage was used as the matrix. The mode of the first amplification stage was following: 94°C at 4 min; 35 cycles at 94°C for 30 s, 59°C for 1 min, and 72°C for 1 min; and final elongation was at 72°C for 5 min; the second amplification stage was at 94°C for 4 min; 35 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 30 s; and final elongation at 72°C for 5 min. After amplification the products were hydrolyzed with restriction endonucleases *Bst*nI. The hydrolysis products were separated by electrophoresis in 3% agarose gel with ethidium bromide, 10 μL of PCR product and 2 μL of loading buffer (ALC Praymteh, Belarus) were applied per lane. Electrophoresis was performed for 2 h at a 100 V. In the reaction, the restriction endonuclease *Bst*nI hydrolyzes the DNA fragment of the A genome, which carries the wild-type *FAD3A* allele and forms 28-bp and 311-bp hydrolysis fragments; the mutant allele (339 bp) is not hydrolyzed. In the C genome, the allele sequence of the wild-type hydrolyzed, forming 174-bp and 36-bp restriction fragments; the mutant allele (210 bp) is not hydrolyzed.

The mutant allele frequency was calculated as the ratio of the number of individual plants of the single form in which the mutant allele was detected to the total number of plants of the analyzed form.

Analysis of the *FAD3A* and *FAD3C* alleles was also performed by the SNaPshot method according to Mikolaiczyk et al. [12]. In order to reduce the cost of

Table 2. Frequency of mutant *FAD2* and *FAD3C* alleles in spring and winter rape

Sample	Type, origin	The frequency of mutations in *FAD2* gene, %	The frequency of mutations in *FAD3C* gene, %
Almaz	Winter[1]	100.0	0
Antei	Winter[1]	100.0	0
Magnat	Winter[1]	100.0	0
Smak	Winter[1]	87.5	0
Pramen	Winter[1]	81.8	0
Gedemin	Winter[1]	71.4	0
Vodolei	Winter[1]	70.0	0
Kroman	Winter[1]	66.7	0
Yawar	Winter[1]	42.9	0
Germes	Winter[1]	40.0	0
Neman	Winter[1]	0	0
Rubin F1	Winter[1]	75.0	0
gr. 7304	Winter[1]	80.0	36.0
Podmoskovnii	Winter[2]	100.0	10.0
Vikros	Winter[2]	100.0	100.0
Dobrodey	Spring[1]	90.0	0
Martyn	Spring[1]	60.0	0
Mayak	Spring[1]	100.0	40.0
Arsenal	Spring[1]	100.0	20.0
VIK2	Spring[2]	100.0	10.0
Severyanin	Spring[3]	100.0	50.0

Variety, hybrid, accession of breeding: [1] The republica unitary enterprise Research and Practical Center of National Academy of Sciences of the Republic of Belarus for Arable Farming; [2] All-Russia Williams Fodder Research Institute; [3] Research Institute of the Northeast and Falensky Selection Station.

the analysis, initial primers were modified by elongation of the poli(A)-structure to 34 bp for mutA-lf and 46 bp for mutC-45F. A high-density DNA length marker for S450 fragment analysis was also used (NPO Syntol, Russia) [14]. The size of the detected fragments was as follows: *FAD3A* (*fad3a*) was 62 bp, and *FAD3C* (*fad3c*) was 66 bp. Electrophoresis of the SnaPshot products was performed with an ABI Prism3500 genetic analyzer (Applied Biosystems, United States).

The fatty acid composition of the oil was determined by gas chromatography according to GOST 51483-99 [15]. The conditions of chromatography were as follows: gas chromatograph, model 3700 (PCG Granat, Russia), glass column with a length of 2 m, inner diameter of 3 mm; solid support of inerton AW-DMCS, fraction of 0.25–0.315 mm. The stationary phase was polyethylene glycol adipate, 15% of the weight of solid support; the carrier gas was helium, and the carrier gas flow rate was 30 cm^3/min. The hydrogen flow rate was 30 cm^3/min; the air flow rate was 300 cm^3/min; the evaporator temperature was 250°C; the detector temperature was 250°C; the thermostat temperature was 185°C; and the sample volume was 1 μL.

RESULTS

In order to identify the *FAD2* allele of *Brassica napus* L. (A genome of *Brassica rapa*, linkage group no. 5) determining an increased content of oleic acid in rapeseed oil, we tested a collection that included 21 samples of Belarusian and Russian spring and winter rape.

Allele-specific PCR enabled the identification of the mutant *FAD2* allele (430 bp) in 14 cultivars, 1 accession, and 1 variety-line hybrid of spring rape and 4 varieties of winter rape based on the presence of the amplified fragment. We established that the frequency of the mutant *FAD2* allele varied from 0 (the Neman variety) to 100% (Almaz, Antei, Magnat varieties) in Belarusian spring rape cultivars. In the variety-line hybrid Rubin, the frequency was 75% (Table 2). For the accession gr. 7304, this value was 80%.

In winter Belarusian rape varieties, the mutation frequency for the *FAD2* gene varied from 60 (grade Martyn) to 100% (grades Mayak, Arsenal); in winter

Table 3. Fatty acid composition of rape seeds of accession gr. 7304, depending on the allelic composition of *FAD2* and *FAD3* genes

No. of the sample	Acids, %							
	palmitic	stearic	oleic	linoleic	linolenic	arachidonic	eicosenic	erucic
Germes variety	4.08	2.5	58.15	19.86	10.76	0.49	1.17	0
gr. 7304*	5.88	3.19	61.6	19.28	6.22	0	0.69	0
11**	6.35	3.19	57.40	20.98	6.95	0	0.59	0
12	5.55	2.71	54.69	24.13	7.38	0	0.82	0
33	5.16	2.11	63.68	18.93	6.52	0	0.67	0
35	4.77	2.46	65.92	16.90	6.44	0	0.72	0
37	6.17	3.52	57.04	22.25	6.29	0	0.58	0
38	6.23	3.96	55.75	22.06	6.09	0	0.81	0
39	5.86	3.80	60.50	18.32	5.18	0	0.82	0
40	4.97	3.26	61.96	19.27	6.09	0	0.78	0
43	6.62	3.33	63.76	16.93	5.03	0	0.61	0
44	5.22	2.37	60.05	20.50	7.30	0	0.72	0
45	5.32	2.72	70.68	14.11	4.67	0	0.63	0
46	5.76	3.18	64.58	15.73	5.35	0	0.79	0
47	4.37	2.27	65.57	17.51	7.11	0	0.60	0
48	5.08	2.67	62.27	19.75	6.74	0	0.65	0
49	5.47	2.88	62.13	19.04	4.98	0	0.68	0
50	5.37	3.40	59.56	21.84	6.23	0	0.61	0

* The average value of 2011.

** Numbers indicate individual plants obtained in 2012 as the result of self-pollination of accession gr. 7304.

and spring, the mutation frequency of Russian rape varieties was 100%.

We also performed an analysis of the collection with the use of DNA markers of a mutant *FAD3C* allele [16]. Among all of the investigated spring varieties of rape and variety-line F1, it was found for the Belarusian Rubin hybrid that the mutant *FAD3* allele was detected only in individual plants of accessions gr. 7304 with a frequency of 36%. In winter Belorussian rape varieties, the mutant *FAD3C* allele was most often detected in the spring Vikros (40%) variety. In the analyzed Russian rape varieties, the mutant *FAD3C* allele was most frequently detected in the spring Vikros variety (100%) and the winter Severyanin variety (50%) [17].

Thus, the use of DNA markers for mutant *FAD2* and *FAD3C* alleles showed a high mutation rate in the studied genotypes for the *FAD3C* gene, with 80–100% of the mutation of the *FAD2* gene, was detected in the spring Vikros variety (RF), spring rape accession gr. 7304 (RB), and winter Severyanin (RF) and Mayak (RB) varieties. The biochemical data support an increased level of synthesis of oleic acid and a decreased level of synthesis of linolenic acid in spring rape accession gr. 7304 as compared with the standard Germes variety (Table 3).

We conducted self-fertilization of 16 individual plants of accession gr. 7304 and performed biochemical and molecular genetic analysis with *FAD2* and *FAD3C* markers [2] (Table 3). Biochemical analysis of the fatty acid content in the self-pollinated rape plants of accession gr. 7304 after thrashing revealed in the investigated individuals with a mutation in *FAD2* gene that the content of oleic acid was higher than in the Germes variety. The maximum value was observed in three individual plants of accession nos. 35, 45, and 47. The synthesis of linolenic acid in accession gr. 7304 was reduced in comparison with the standard Germes variety (Table 3) and Belarusian varieties of spring rape [1]. The lowest values were observed in plant nos. 45 and 49.

Since it is known that the fatty acid composition of rape seeds varies depending on the allelic composition of *FAD2* and *FAD3* and the ratio of mutant *fad2*, *fad3a* and *fad3c* alleles and wild-type *FAD2*, *FAD3A* and *FAD3C* alleles [18], we developed DNA-markers in order to identify the alleles determining the synthesis of linolenic acid and to detect genotypes with its reduced synthesis. This allowed the differentiation of mutant alleles and wild-type alleles of *FAD3* genes of the A and C rape genomes. For this purpose, the contrasting linolenic acid genotypes no. 45 (low content of linolenic acid, high content of oleic acid) and no. 44 (high content of linolenic acid, low content of oleic acid) were selected. These genotypes were obtained as a result of self-fertilization of individual plants of accessions gr. 7304, which is used for the development of molecular markers

We have developed a method to detect the *FAD3* alleles of the A and C genomes of *Brassica napus* [13], including:

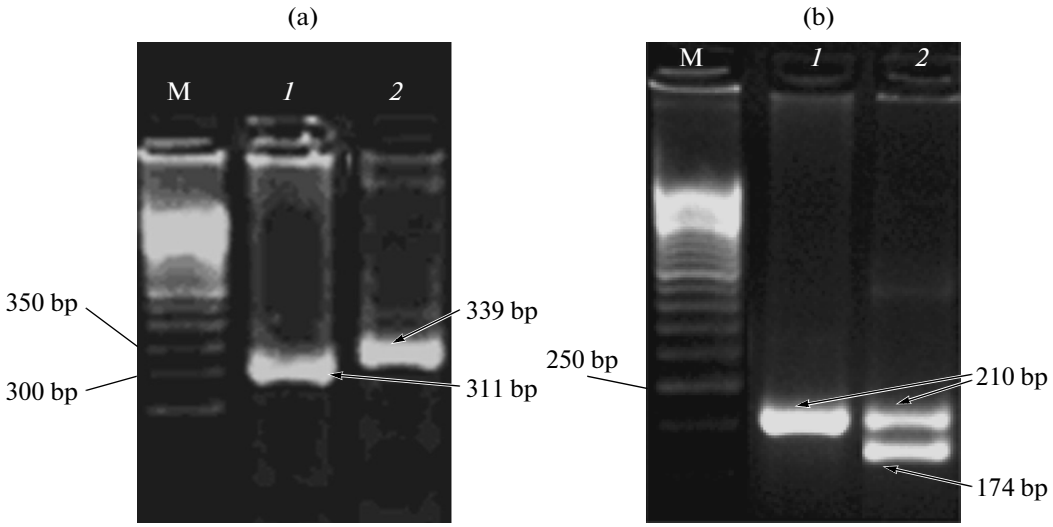

Fig. 1. Electrophoregram of restriction products of PCR fragments obtained by DNA amplification of rape genotypes with different linolenic acid content with allele-specific dCAPS-primers to genes of A genome (a) and C genome (b) of *Brassica napus*. *1*—no. 44 (genotype with increased content of linolenic acid), *2*—no. 45 (genotype with decreased content of linolenic acid); M—molecular weight marker M 50 bp (ALC Praymteh). Arrows indicate unique marker.

(1) independent amplification of *FAD3* gene fragments of the A and C rape genomes with the use of gene-specific primers;

(2) the introduction of single-nucleotide substitutions associated with the wild-type allele nucleotide in the amplified sequences of *FAD3* genes of the A and the C genomes;

(3) restriction of the amplification products and differentiation of the wild-type alleles and mutant *FAD3* alleles of the A and C genomes.

During the restriction reaction, the restriction endonuclease hydrolyzes the DNA fragment of the A genome, which contains the wild-type *FAD3A* allele. This forms restriction fragments of 311 and 28 bp (a DNA fragment of 339 bp obtained from samples carrying the mutant allele was not hydrolyzed). The C-genome sequence containing the wild-type allele forms restriction fragments of 174 and 36 bp (a DNA fragment of 210 bp obtained from samples carrying the mutant allele was not hydrolyzed) (Fig. 1).

Individual plants (no. 45 and no. 44 of accession gr. 7304) were also analyzed with DNA markers for *FAD3A* and *FAD3C* genes by SNaPshot (Fig. 2). It was revealed that plant no. 45 is a recessive homozygote for the *FAD3A* gene and heterozygous for the *FAD3C* gene, in contrast to plant no. 44 (Fig. 2). Thus, the SNaPshot data confirmed our results (Fig. 1), as did the data from biochemical analysis by gas-liquid chromatography (Table 3).

We obtained plants of the F_2 second generation by crossing high oleic acid and low oleic acid plant no. 45 of accession gr. 7304 of Germes and Antei varieties. Plants of the F_2 generation (185) were analyzed with DNA markers for mutant *FAD2* alleles (Fig. 3). The frequency of the mutant allele in the Germes generation gr. 7304 was 93.8%. In the F_2 generation of Antei X gr. 7304, no. 45 was 100%.

Using the produced markers for *FAD3* genes of the A and C rape genomes, we established that all of the individual plants of the F_2 Germes × gr. 7304 no. 45 (90 pcs.) and F_2 Antei × gr. 7304 no. 45 (94 pcs.) were homozygous for the mutant allele of the *FAD3A* gene and heterozygous for the *FAD3C* gene (Fig. 4), with the exception of one individual plant of the F_2 Germes × gr. 7304 no. 45, which is a double mutant for the *FAD3A* and *FAD3C* genes (Fig. 4, *6*, *13*). Individual plants selected by DNA markers carrying mutant *FAD3A* and *FAD3C* alleles are currently used in the selection process.

DISCUSSION

Relatively high levels of α-linolenic acid (about 10%) were detected in the rapeseed oil, leading to its rapid oxidation [18]. Rapeseed oil that is perfect for human nutrition contains less than 3% of linolenic acid [1]. Chemical mutagenesis, together with classical breeding methods, is used to improve the quality of oil and the production of new varieties with an optimal fatty acid composition in such crops as sunflower, soybean, and rape. The first chemical mutagenesis was applied to the Canadian rape variety Ogo with the use of the M11 mutant line. A low content of C18:3 (3%) was obtained [18]. The low oleic varieties Stellar and Appolo were produced as a result of recombinant crossings of the M11 line [19, 20]. A series of such lines was later obtained via chemical mutagenesis. In particular, Dow AgroScience (United States) produced the DMS100 mutant line based on the AG019 line [21]. A mutant line of winter rape was produced by

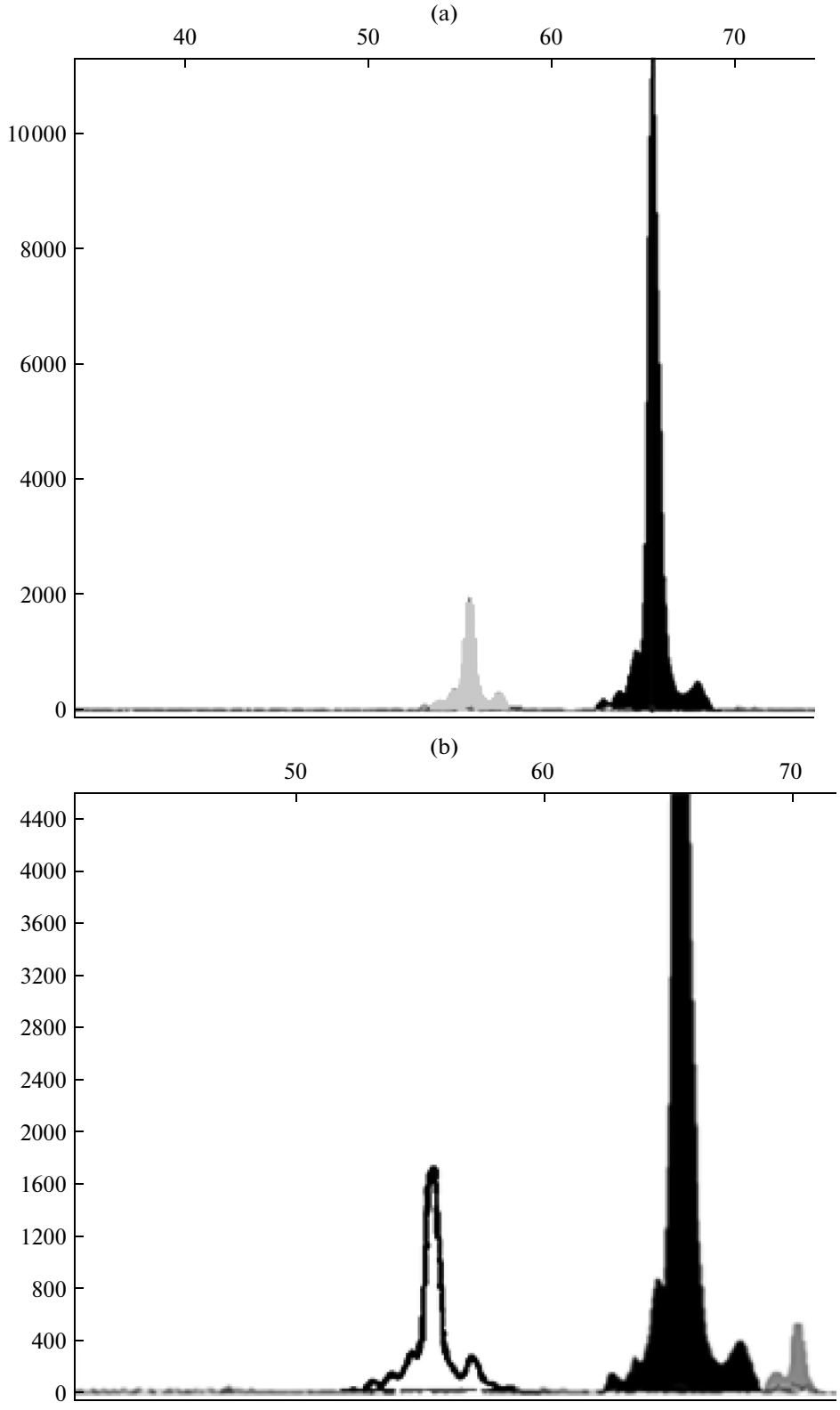

Fig. 2. Detection of *FAD3A* and *FAD3C* alleles by SnaPshot method. (a) no. 44 (genotype with increased content of linolenic acid), (b) no. 45 (genotype with decreased content of linolenic acid); wild-type allele of *FAD3A* gene—speckle, the mutant allele of *FAD3A* gene—white (C-to-T substitution); wild-type allele of *FAD3C* gene—black, mutant allele of *FAD3C* gene—grey (G-to-A substitution).

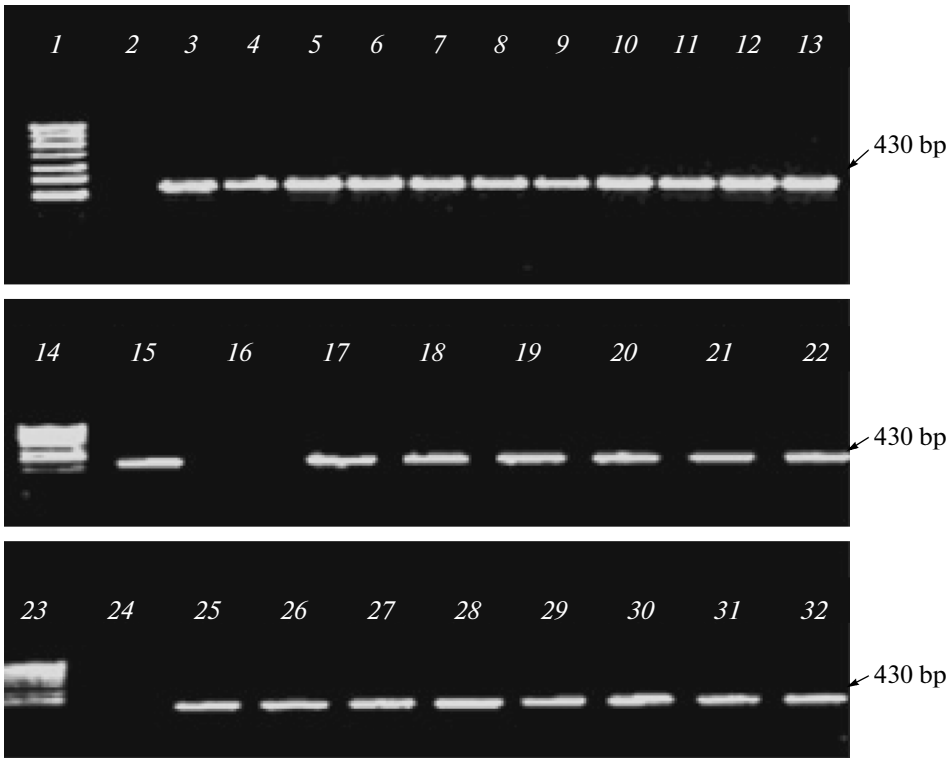

Fig. 3. Electrophoregram of amplification products of genomic DNA of individual F_2 plants (Hermes × gr. 7304 no. 45) with primers to a mutant allele of the *FAD2* gene. *1, 14, 23*—molecular weight marker M100 (ALC Praymteh).

Spasibionek [22] and used to produce stable inbred lines with a high oleic acid content (≥75%) and a low linolenic acid content (≤3%).

The evaluation of rapeseed oil is conventionally performed by standard biochemical methods to determine the fatty acid composition of the oil in the seeds. Gas-liquid chromatography is highly informative for the evaluation of the level and ratios of fatty acids in the seed oil [6]. However, this method does not allow the identification of homo- and heterozygous genotypes. In the genes that determine the level of oleic and linoleic unsaturated fatty acid synthesis, the distribution of these genes in the heterozygous state will occur as a result of pollination and phenotypically will manifest in the next generation. In addition, this method does not allow the selection of parental genotypes for hybridization in the current generation, since the analysis of a selection of material is carried out only after the thrashing of seeds.

The scientific data accumulated to date confirm the significance of the effect of mutations in *FAD2* and *FAD3* with an increased oleic acid level and a decreased linoleic acid level in the seeds of *Brassica napus* L. [11, 20]. Therefore, studies should be directed towards the search for optimal methods of identifying genotypes with an increased oleic content and a decreased linolenic unsaturated fatty acid content in the large rape populations used in the breeding process. The use of molecular markers enables analysis directly at the DNA level and makes it possible, with proper selection, to avoid the disadvantages of the conventionally used gas-liquid chromatography method.

Molecular markers associated with an increased oleic content and decreased linolenic unsaturated fatty acid content were developed previously [2, 21]. Schierholt et al. [23] identified three AFLP markers associated with a mutation causing an increased synthesis of oleic acid in winter rape (*Brassica napus* L.). RAPD and RFLP markers associated with low levels of linolenic acid were developed [24–26]. However, these markers either have low resolution or are not suitable for large-scale screening. High-resolution SNP markers were developed by Hu et al. [2, 21] and Milcolaiczyk et al. [12] for the detection of *FAD2* and *FAD3* mutations.

The goal of our study was to analyze the collection of varieties, accessions, and variety-line hybrids of spring and winter rape of Belarusian and Russian selections with the use of DNA markers for the *FAD2* and *FAD3* genes in the selection of genotypes with a low linolenic acid content and their involvement in the selection process.

During the stage of the primary selection, the collection was screened with DNA markers for mutant *FAD2* and *FAD3* alleles developed by Hu [2]. Genotypes with a high frequency of mutations of these genes include the spring Vikros variety (RF), the spring rape accession gr. 7304, and the winter Severyanin (RF)

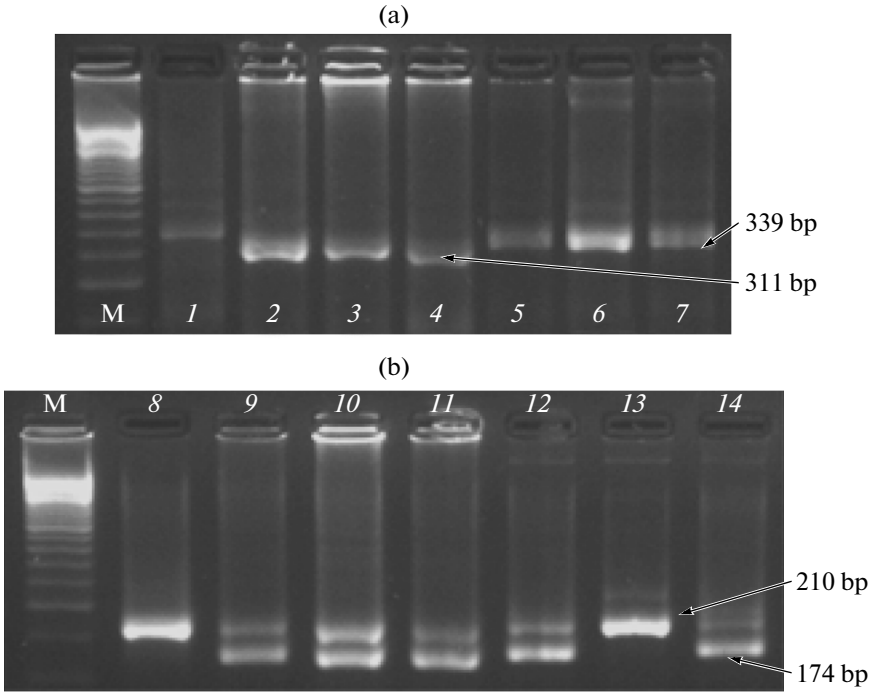

Fig. 4. Electrophoregram of restriction products of PCR fragments obtained by DNA amplification of individual F_2 plants with allele-specific dCAPS- primers to *FAD3* genes. (a) detection of the mutant *FAD3* allele of A genome, (b) C genome; M—molecular weight marker M50 (ALC Praymteh); *1, 8*—control (A and C genomes, respectively); *2–4* (*9–11*)—individual F_2 plants obtained by crossing Antei variety and gr. 7304 no. 45. *5–7* (*12–14*)—individual F_2 plants obtained by crossing Germes variety and gr. 7304 no. 45.

and Mayak (RB) varieties. They were selected based on the results of this screening.

We later developed dCAPS (Derived Cleaved Amplified Polymorphic Sequences) markers and an identification method for *FAD3* alleles controlling the linolenic acid level in rapeseed oil to differentiate mutant alleles and wild-type *FAD3* alleles of the A and C genomes. The method includes independent amplification of *FAD3* gene fragments of A and C rape with the use of gene specific primers, allele-specific primers, and the restriction of amplification products. This method has a lower cost as compared to the previously developed, precise but costly SNaPshot method for the detection of *FAD3* genes of A and C rape genomes [12].

Allele-specific DNA markers developed by us for the *FAD3* genes controlling the synthesis of linolenic acid in rape seeds have a number of advantages over the traditionally used biochemical analysis of the fatty acid composition of the seeds, including the possibility of direct identification of the mutant and wild-type *FAD3* alleles of A and C genomes in the breeding material during the early stage of plant development, which makes it possible to discard material that is undesirable for breeding at the beginning of the growing season and to rule out biochemical analysis of the fatty acid composition of the oil in breeding samples after the harvesting and threshing of seeds. These markers make it possible to save time and money on the handling of crops, harvesting, and the postharvest processing of breeding material and to test for the *FAD3* gene localized in the A and C rape genomes independently. This allows a twofold reduction in the time required for the production of varieties. The use of allele-specific dCAPS-primers enabled identification in F_2 generation individual plants homozygous for *FAD3A* and/or *FAD3C* genes, which are currently used for further selection and breeding in the production of new varieties with a reduced linolenic acid content in the oil.

However, it should be noted that the exact number of functional loci of *FAD2* and *FAD3* genes of A and C genomes that affect the levels of oleic and linoleic unsaturated fatty acids is not completely defined. In 2012, Yang et al. [10] performed a detailed bioinformatics analysis and cloning of *FAD2* and *FAD3* genes of A and C genomes. This analysis revealed additional alleles in the A and C genomes that may influence the oleic and linoleic unsaturated fatty acid contents. Therefore, further study of these loci is required in order to use the results in the selection process for the production of new rape varieties.

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