Bulgarian Journal of Agricultural Science, 29 (No 6) 2023, 1079–1089

Mutagenesis as tool for enhancement of fatty acid composition of rapeseed *(Brassica napus* L.)

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Abstract

Marcheva, M., Petkova, M. & Atanassova, S. (2023). Mutagenesis as tool for enhancement of fatty acid composition of rapeseed (*Brassica napus* L.). *Bulg. J. Agric. Sci., 29*(6), 1079–1089

Stable genetic improvement of the fatty acid composition of rapeseed (*Brassica napus* L.) was a prerequisite for promoting and expanding its industrial applications. Irradiation with 10 and 15 krad gamma rays ^{60}Co of seeds of two registered varieties – Trabant and Abacus provoke genetic and biochemical changes in the mutant generations of rapeseed. The induced mutants were reproduced for three years and compared with control plants in the experimental field of Agricultural University–Plovdiv. Each mutant was isolated and self-fertilized. Biometric characteristics were described for thirty plants in three replicate each year and variant. The initial genotypes responded to the irradiation with various changes in the plant height, branching, number of siliques per plant, seeds per silique and seed weight per plant. The quality and quantity of fatty acids content were screened by gas chromatography mas spectrometry (GC/MS) and near-infrared reflectance spectroscopy (NIRS). Spectral data were analyzed by principal component analysis (PCA) and partial least square regression (PLS) was used for quantitative analysis. The biochemical analyses of the mutants showed lower content of mono-unsaturated fatty acids as oleic acids and higher content of polyunsaturated fatty acids as linoleic (C18:2) and linolenic acids (C18:3). The changes in the fatty acids' composition correlate with a lower plant height and better branching of the plants. Irradiation with 100 Gy led to the creation of mutants with larger seeds and higher production potential per plant. The mutants could be used for further plant breeding procedures for enhancing the productivity and quality of rapeseed oil as a valuable source for multiple industrial and food purposes.

Keywords: rapeseed; Brassica napus; mutagenesis; fatty acid; gas chromatography; NIRS

Introduction

In Bulgaria, rapeseed (*Brassica napus* L) is the second most important oil crop after sunflower (*Helanthus annuus* L.) and, in addition to household needs. Rapeseed oil was also used as an energy raw material. In the region there were favorable conditions for the cultivation of rapeseed and the economic importance of the culture was growing steadily because of increased consumption of vegetable oils and protein. Besides traditional uses in the food industry, rapeseed was used to produce biodiesel. The distribution of biodiesel as a component of an environmentally safe fuel leads to a growing demand for rapeseed and a lasting increase in its purchase price.

Rapeseed's biological and chemical value consists of the high protein and essential fatty acids content. Seeds of different varieties contain about 40–45% oil, which provides raw materials to produce vegetable oil for domestic needs, as well as methyl esters (biodiesel), industrial lubricants and hydraulic oils, detergents, soaps and rapidly degradable plastics (Friedt et al., 1999). After extraction of oil by cold pressing, debris contains 20–34% high-quality protein, which was used for livestock fodder. In addition, it may be pointed out that variations in protein composition in different species were larger than the variations in the composition of fatty acids. Rapeseed oil has a low concentration of saturated fatty acids – 7%, and a high concentration of unsaturated fatty acids and beneficial omega-3 fatty acids (Eskin et al., 1996). As evidenced by this data, it was clear that rapeseed oil has low oxidative stability due to the high content of unsaturated fatty acids, linoleic acid, and linolenic acid. A negative aspect of the hydrolysis of unsaturated acids is their decomposition into trans-fatty acids. The nutritional value of rapeseed oil was compromised by the presence of erucic acid and glucosinolates. The presence of glucosinolates above a specific limit makes meals poisonous for animals. This required developing methods of genetic modification of rapeseed varieties and the obtained ones (a double zero) were characterized by a reduced content of erucic acid and glucosinolates. Through antisense inhibition of oleate (18:1) oil was obtained that contains more than 80% oleic acid and significantly reduced amounts of polyunsaturated fatty acids (Della Penna, 1995; Hitz et. al., 1995). Mono-unsaturated fatty acids were more resistant to oxidation and were more healthy components in the human diet. Some projects work on the enrichment of rape with other substances, such as lauric acid and vitamin A.

Altering the fatty acid composition of rapeseed oil and increasing its value as food and industrial raw material was a key objective in the breeding programs of the Brassica genus worldwide. The traditional approaches to changing the composition of fatty acids include the study of spontaneous or induced mutations occurring in the plant species or closely related species. These mutations have shown that several enzymes were related to the biosynthesis of fatty acids (Weselake et al., 2008). Through conventional plant breeding and genetic techniques, many new varieties have been developed, containing oil with significantly altered levels of fatty acids with long (erucic acid) and medium chains (oleic and linoleic acid) for different applications. Due to the impossibility to use recombinant DNA technology in our country, the study of spontaneous and induced mutation for the change of the fatty acid biosynthesis in Brassica species remains the only available option for modifying the composition of fatty acids (Schierholt et al., 2000). Many of these fatty acids were valuable raw food, while others (such as stearic and palmitic acids) contribute to a raised cholesterol rate and an increased risk of cardiovascular disease. Their reduction in the seeds of oil plants was a major problem underlying the genetic and breeding programs on rapeseed. This project, based on the current state of the breeder-genetic methods, offers approaches to modify the quantitative and qualitative composition of rapeseed oil to its wider use as a health food and biofuel.

Since the cultivation of rapeseed in the EU was conventional, the use of unmodified rapeseed was preferred in the food and fodder industry. The development and the widespread use of various molecular markers, as well as gas chromatography and infrared spectrometry, enable the control and analysis of the quality and fat content of seeds (Havlíčková et al., 2014; Abd Elsalam et al., 2014; Nagaoka and Ogihara, 1997). With the improvement of tools and methods in genetics and biotechnology, the question arises to produce new and useful fatty acids.

The change in the composition and content of C18 unsaturated fatty acids was one of the most important tasks in the selection of oilseed rape. Induced mutagenesis was a powerful tool for creating new rapeseed genotypes. When exposed to gamma rays, the variation of the obtained genetic changes was with high frequency and a wide range of morphological and biochemical changes. The present study examines genetic and biochemical changes resulting from irradiation with 10 and 15 krad gamma rays hybrid When the seed quality trait was determined by the genotype of the embryo it was useful to have NIRS calibrations for single seeds to enable selection among segregating F2-seeds (Velasco and Mollers, 2002). Such single NIRS calibrations have been developed for determining fatty acids (Oblath et al. 2006).

The modification of the fatty acid composition of seed oil to develop new genotypes having alternative oil characteristics has been an unimportant objective in quality breeding in rapeseed, and it is required to determine the fatty acid composition of the oil in a large number of breeding lines. GC/MS analysis is time-consuming, expensive, and destructive; therefore, they are not adequate for selecting superior lines from a number of rapeseed germplasm lines. Thus, a rapid and nondestructive method such as NIRS is in high demand to evaluate oil quality for rapeseed breeding programs.

The current report was presented on the genetic variation of individuals and total fatty acid content in registered varieties of rapeseed and their gamma-radiation mutants using GC/MS and NIRS. The identification of genotypes with high oleic acid and low unsaturated fatty acid contents was very important. The development and introduction of NIRS

calibrations suitable for an efficient and non-destructive selection in breeding programs in Bulgaria were discussed. The current report was presented on the genetic variation of individuals and total fatty acid content in registered varieties of rapeseed and their gamma-radiation mutants using GC/MS and NIRS.

Materials and Methods

Plant material

Seeds from rapeseed genotypes Trabant and Abacus, differing in the composition of fatty acids with 18 carbon atoms – oleic, linoleic and linolenic, were used as initial material. The seed sample was divided into four – a control and three variants of irradiation with gamma irradiation with 5 krad, 10 krad, and 15 krad gamma-ray ^{60}Co to induce mutations. The germination rate of all variants in M1 was calculated and morphological changes of M1, M2 and M3 generations were analyzed. Although oilseed rape was considered a self-pollinating plant species (Williams et al., 1986), insect pollination can further increase yield and quality (Bommarco et al., 2012). Proper research of its progeny required the isolation and self-fertilization of each plant in M1 and M2 and cultivation in separate plots of inbred mutants.

Field trials were established on leached chernozem soil in the experimental base of the Agricultural University–Plovdiv. Traditional for the region crop production technology has been used. No significant environmental or biotic stress factors were observed during the vegetation of the rapeseed in 2018 – 2021 (42° 8′ 9.9492′′ N, 24° 44′ 31.8048′′ E).

To determine the variability of the elements of the productivity – plant height (cm), number of branches, number of siliques per single plant, number of seeds per one silique, seed weight per plant (g), they were measured thirty normally developed till maturity and with good agronomical value M1 plants. Plants which formed only leaves, with severe deformations during vegetation and early death or lack of flowers or siliques were not analyzed. Isolation and inbreeding of each plant were provided. After analyzes of the seed production of M1 plants with visual changes, the best of them were propagated in the M2 generation. The M3 generation was formed by the seeds of the most productive and suitable for agronomical uses of M2 plants. Large number of plants (of approx. 20 000 to 80 000 from each genotype and irradiation treatments) were monitored and the elements of productivity of selected 30 plants per variant with good agronomic value were analyzed (figure 3). The arithmetic means and their errors, the accuracy indicators and the coefficients of variation were calculated. The significance of the differences between the variants was assessed. Statistica 7.0 software was used for the statistical evaluation (Stat Soft Inc. 2004). Raw data were processed by the *t*-test (dependent samples), and cluster analysis was performed for the grouping of the studied sampling sites based on studied biometrical and physiological parameters. Relationships between the studied parameters in collected leaf samples were tested using Pearson correlated coefficients. All the analyses were significant at $p < 0.05$.

Seeds Germination

The dry seeds of two *Brassica napus* L. cultivars (Trabant and Abacus) M1 generation irradiated with doses of Gamma rays 5, 10, and 15 krad were tested for germination rate. The irradiated seeds in addition to the non-irradiated seeds (control) were surface sterilized by immersion in ethanol 70% for 5 s. followed by immersion in sodium hypochlorite 3% (v/v) for 20 min and a few drops of tween-80 then rinsed in sterile distilled water for three times. Sterilized seeds were germinated on 0.8% agar (w/v) then they were incubated at 25°C under 16/8h day/night photoperiod (1000 LUX). Each test includes five jars with five seeds in three replicates for each genotype and treatment. Germination percentage (%) was calculated for the treated and non-treated seeds (control) as follows: number of germinated seeds G P (%) = Number of germinated seeds/total number of seeds × 100.

GC-GC/MS

The seeds of the hybrids Trabant and Abacus (2 g), as well as seeds of isolated inbred plants with a good agronomical value of the radiation-treated variants, were studied for the quantitative and qualitative composition of fatty acids.

The fat content was determined by the residual method with a Soxhlet apparatus. One gram of mature seed of each sample was oven dried for 4 h at 70°C and then was milled in a coffee grinder with 3.5 g diatomaceous earth (Haagenson et al. 2010; Petkova et al. 2015). Extractions were performed at 100°C, 6.7 MPa with a 5 min equilibration time and three 10 min static cycles having a 100% flush volume and 60 s purge time. The solvent containing extracted oil was collected in pre-weighed vials, and solvent was evaporated to dryness with a stream of dry air (-70°C dew point). Extracted samples were air dried, and reground for a second extraction and the total oil recovery from the two extractions was recorded. Oil is reported as a percent of seed dry weight.

The extraction of fatty acids from the seeds was performed with a solution in 1 ml of esterification buffer (75 ml of hexane, 20 ml of chloroform and 5 ml of sodium methoxide in methanol) (Zlatanov et al., 2009). Samples of 0.1 to 0.3 g of dried seed were ground in a mortar and pestle and vortexed in 0.5 to 2 ml of hexane -chloroform-sodium methoxide derivatization reagent to produce fatty acid me-

thyl esters (Petkova et al., 2015). GC analyses were performed with a Hewlett-Packard gas chromatograph (model: HP5890fitted with a 30 m FFAP capillary column according to Zlatanov et al., 2009 (0.25 mm narrow aperture and 0.5 μm thick film).

Spectral measurements

The spectral measurements of the tested samples were made using the NIRQuest 512 spectral apparatus (Atanasova et al., 2007; Guo et al., 2021) . It was a portable scanning spectrophotometer operating in the range of 900-1700 nm. It was shown in Supplementary figure 1. It represents a new generation of spectrophotometers working with optical fibers and a diode line with 512 pixels as a detector. Spectral data of 512 wavelengths in different formats – intensity, absorption, reflection, or transmission coefficients were obtained from the device. Spectral measurement of each of the samples was made non-destructively by measuring diffuse reflection from whole seeds. In the present study, a reflection measuring attachment was used, shown in Supplementary figure 1, consisting of 6 optical fibers, through which the analyzed sample was illuminated, and one, through which the received signal was returned to the spectrophotometer. Between five and seven spectra were obtained for each sample, and the radiation was focused on different seeds.

The Pirouette 4.5 program (Infometrix, Inc., Woodinville, WA, USA) was used to process the spectral data, through which quantitative analysis and classification were performed using various mathematical methods. The SIMCA (Soft Independent Modeling of Class Analogy) method was used to classify the samples based on their spectra. In this method, the samples were divided into classes depending on some parameter. A model was then made for each class by analyzing the main components. Partial least square regression (PLS) was used for quantitative analysis. This method processes spectral data and the value of the required quantitative parameter. New factors were calculated, based on the first factor, which describes the maximum part of the variations in spectral and quantitative data. The second factor describes the maximum part of the state of variation, etc. Thus, both spectral and sample information can be described using these two factors (Hom et al., 2007) [18].

Results and Discussion

Morphological variability

Irradiation with the lowest dose of 5 krad gamma rays ^{60}Co did not provoke significant changes in the germination rate of the treated seeds (Table 1). The M1 plants were typical for the genotype without significant differences in the morphological traits and the elements of productivity. No further changes were proved in M2 and M3 generations. We consider that no mutations were induced and no comments on this variant were made. Germination rates of the seeds, irradiated with 10 and 15 krad gamma rays ^{60}Co were close to 50%. Their progenies revealed considerable phenotypic variations, which were discussed in detail below.

Table 1. Germination rate of control and irradiated seeds of rapeseed varieties Trabant and Abacus

Genotype	Number of treated seeds, %	Germination, %
Trabant – control	100	94
M1 – 5 krad	100	95
M1 – 10 krad	100	67
M1 – 15 krad	100	48
Abacus – control	100	92
M1 – 5 krad	100	91
M1 – 10 krad	100	63
M1 – 15 krad	100	52

A significant change was observed in all characteristics. Some of the plants showed varying degrees of leaf deformation and stem distortion (Figure 1). They were cases of discoloration, altered flowers or pollination problems. The elements of productivity as branching, number of siliques per plant and seed weight per plant were affected to a greater extent by irradiation as shown in Tables 2 and 3. The plant height remained relatively stable within the mutation generations of the variety Trabant but in most cases, mutants were shorter than the initial genotype (Table 2). Over the applied gamma radiation doses, results indicated no significant differences between the three investigated cultivars in M2 and M3 generations of Trabant and Abacus, whereas M1 of Abacus (M1 – A10 krad) showed the longest height of 149.56 cm when compared to control plants.

The reaction of the Abacus to the irradiation was different and shown separately in table 3. The plants in the M1 generation, treated with a higher dose – 15 krad gamma rays, were much shorter (50%) than the control population. The smaller dose provoked statistically significant but small changes in the trait. In the M3 generation, both variants were only 22–23% shorter than the standard. The shortest plants were noticed for the dose of 10 krad was applied to M2 generation of Abacus (91.51 cm) and 15 krad to M3 generation of Trabant (85.65). The current result was in accordance with Rafiullah and Hasan (1994) who reported that a gradual decrease in plant height was observed as radiation dose increased. There were some reports which showed that the higher exposures to gamma rays were usually inhibitory (Rahimi and Bahrani, 2011).

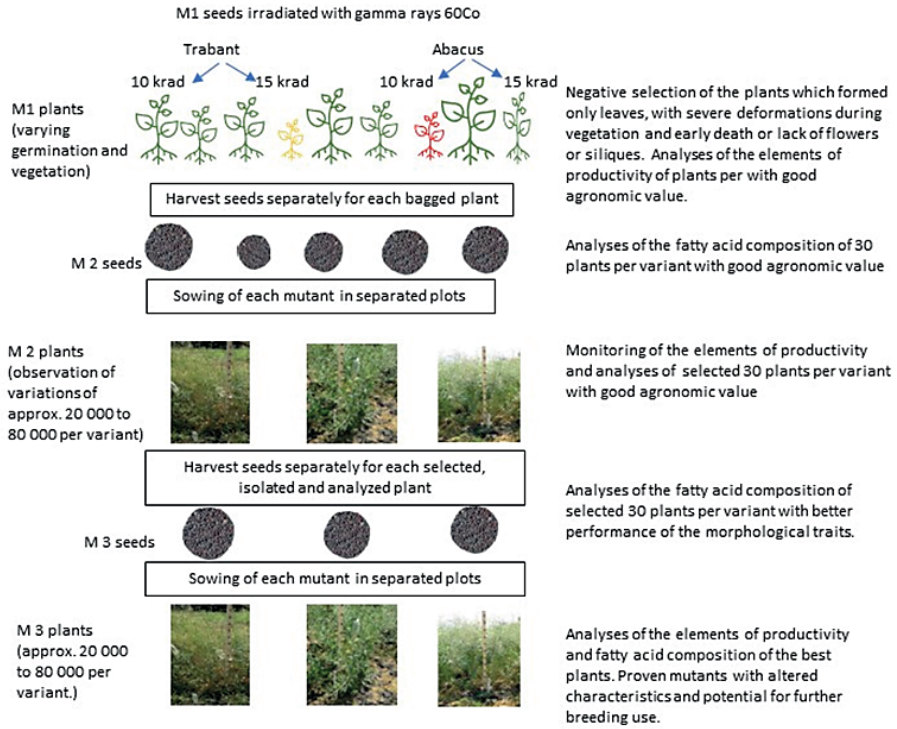

Fig. 1. Flow chart of activities in succeeding mutation generations

An opposite tendency was observed for the branching. Unfortunately, that change was not related to the bigger formation of siliques per plant and in most cases, observed problems in silique formation and fertilization. Therefore, the coefficients of variation (S%) for seed weight per plant reached 26.2% for the higher dose of irradiation. Analyses of the elements of productivity and selection of progenies of plants with better agronomical values showed significant improvement in M3 generation of Trabant, irradiated with both doses of treatment. The number of siliques per plant for the higher dose of irradiation was increased by 39% but the seed weight per plant marked a 90% of improvement. With

Table 2. Variation of the plant height (cm), branching (number), siliques per plant (number), seeds per silique (number), seed weight per plant (g) in variety Trabant and its mutation progenies M1, M2, and M3

Genotype/ traits	Plant height	Branching	Siliques per plant	Seeds per silique	Seed weight per plant
Trabant – control, x̄	147.37***	9.47***	130***	20.56**	6.76**
S%	4.18	8.36	6.47	9.22	9.94
M1 – 10 krad, x̄	126.62***	13.25***	89.68***	25.75***	7.2**
S%	7.29	14.26	14.21	14.70	19.25
M1 – 15 krad, x̄	105.9***	12.18***	45.8***	19.32**	3.92***
S%	15.31	18.12	16.73	17.82	26.3
M2 – 10 krad, x̄	134.75***	12.35***	122.18***	19.37**	7.2**
S%	15.07	16.74	18.59	16.84	19.27
M2 – 15 krad, x̄	101.71***	13.24***	47.35***	21.13***	5.22***
S%	13.51	18.45	18.68	16.74	19.42
M3 – 10 krad, x̄	102.09***	15.38***	198.13***	22.81*	11.12***
S%	7.65	18.04	17.85	20.26	17.82
M3 – 15 krad, x̄	85.65***	10.78***	180.54***	22.67*	12.69***

$P < 0.05$; *** $P > 0.001$

Table 3. Variation of the plant height (cm), branching (number), siliques per plant (number), seeds per silique (number), seed weight per plant (g) in variety Abacus and its mutation progenies M1, M2, and M3

Genotype/ traits	Plant height	Branching	Siliques per plant	Seeds per silique	Seed weight per plant
Abacus – control, x̄	148.25**	13.75*	89.62***	24.18**	15.81*
S%	5.85	5.59	7.82	5.88	7.51
M1 – 10 krad, x̄	149.56*	13.93*	99.37***	23.05**	15.88*
S%	10.46	18.79	19.86	14.55	12.32
M1 – 15 krad, x̄	98.01***	13.83*	33.55***	28.2***	14.8***
S%	11.33	16.4	15.31	19.71	16.73
M2 – 10 krad, x̄	91.51***	7.4***	30.2***	17.6***	10.32***
S%	10.46	18.79	19.86	14.55	12.32
M2 – 15 krad, x̄	122.31***	13.5**	93.92***	21***	15.20**
S%	11.33	16.4	15.31	19.71	16.73
M3 – 10 krad, x̄	136.15***	7.51***	166.24***	26.08***	10.02***
S%	9.8	17.67	19.71	19.84	17.36
M3 – 15 krad,x̄	139.08***	13.48**	117.37***	25.5***	13.07***
S%	11.35	19.54	17.37	18.06	14.09

$P < 0.05$; *** $P > 0.001$

a 42% decrease in the plant height in the same variant and a lower possibility for lodging, the potential for crop production of these plants was promising. Shah et al., (1990) found similar results when they reported that gamma rays increase the number of primary branches in rapeseed.

This genotype remains relatively more stable also regarding the elements of productivity. In M1 generation the coefficient of variation within the mutation populations was larger than in the control plants but differences in the number of branches, seeds per silique and seed weight per plant were small. Only in the variant treated with 15 krad was the formation of siliques per plant severely hampered. The siliques formed then had more seeds in them.

In the next generation, M2, we observed the opposite situation – bad formation of siliques in the variant treated with 10 krad (almost 66% less than the control) with poor fertilization and much lower yield per plant.

The last year of field trials of M3 progenies of Abacus was favorable for good branching and formation of more siliques per plant than the control, (like Trabant M3 generation) but even the large number of seeds per silique in both variants was not enough to ensure good productivity. The seed weight per plant remained below the standard when compared with the results of thousand-seed weight (3.49 and 3.94 g) by Li et al., (2019). In the M3 generation, the coefficient of variation for the elements of productivity was larger than the initial variety and we continue the selection of progenies. Siddiqui et al., (2009) showed that combinations of physical and chemical mutagen have shown a considerable increase in variance for all the traits under study enhancing the effect on primary branches. The induced variation can be exploited in the evolution of new varieties of rapeseed with improved agronomic traits. Along the same line, Souror (1998) obtained four new mutant lines of oilseed rape (*Brassica napus*) by gamma irradiation one of them was with improvement in branches number, silique number and seed weight per plant. Das et al., (1999) obtained two new mutant lines of oilseed rape by gamma irradiation with improvement in oil content and fatty acid composition. Javed et al., (2000) produced five mutants with significantly higher yield than current using different doses of gamma rays (750 to 1250 Gy) with *Brassica juncea*.

Study of total fat and fatty acid composition

The fatty acid composition of the oil from the hybrids Trabant and Abacus, M1, M2, and M3 generation of the same hybrids irradiated with 10 and 15 krad gamma rays was determined. The results of the chemical analysis show that, in general, irradiated hybrid varieties show lower oleic acid content and higher linoleic and linolenic acid content compared to non-irradiated variants. From the data presented in Table 4, there was a tendency to increase the total content of crude fat in the hybrid populations of M1, M2, and M3 of Trabant, compared to non-irradiated plants. This in turn was associated with a decrease in the total content of saturated fatty acids and an increase in the content of unsaturated fatty acids. The percentage of unsaturated fatty acids was the highest (94.2%) in the seeds of M2 T-15, and the content of saturated fatty acids in the oil of variant M1-T15 was the lowest.

Table 4. Total fat content, saturated and unsaturated fatty acids in the seeds obtained from M1, M2, and M3 segregating populations of Trabant and Abacus treated with 10 and 15 krad gamma rays, compared to the non-irradiated baseline Trabant

Crude fat and fatty acid content, %	Trabant	M1-T10 krad	M1-T15 krad	M2-T10 krad	M2-T15 krad	M3-T10 krad	M3-T15 krad
Crude fat content	35.8	36.6	37.2	36.8	46.6	46.6	38.6
Saturated FA	10.5	7.9	7.3	5.3	6.6	5.8	6.3
Unsaturated FA	89.9	84.0	85.2	83.3	81.1	94.2	93.7
	Abacus	M1-A10 krad	M1-A15 krad	M2-A10 krad	M2-A15 krad	M3-A10 krad	M3-A15 krad
Crude fat content	35.9	40.7	39.5	43.2	45.1	42.5	47.1
Saturated FA	10.1	9.3	7.5	6.3	6.8	6.3	6.9
Unsaturated FA	89.7	82.3	83.5	85.2	86.2	92.7	93.7

Table 4 shows a similar inverse correlation between total fat values and saturated and unsaturated fatty acid content. Rapeseed oil from the Abacus hybrid shows a higher content of saturated fatty acids of the M1, M2, and M3 generations compared to irradiated variants. In contrast, the percentage of unsaturated saturated acids was positively affected by gamma irradiation and was significantly high at M3 A1-10 and M3 A-15, 92.7% and 93.7%, respectively.

Table 5 present the results of the percentage of saturated and unsaturated fatty acids with a carbon chain length from C14 to C18 in the oil obtained from seeds of hybrid populations of M1, M2, and M3 of Trabant and Abacus, irradiated with 10 and 15 krad compared to non-irradiated seeds. Lauric acid was not contained in the hybrid Trabant and Abacus but was present in low concentrations of 0.1–0.2% in the oils of the other segregated M1 to M3 generations. The amount of myristic acid C14:0 does not exceed 0.2% in all tested hybrids, which was harmless to human health. Polyunsaturated fatty acids include oleic, linoleic and linolenic acids. Oils rich in oleic acid (C18:1) were suitable were heat treatment and long shelf life. In the present experiment, there was a decrease in the content of oleic acid in the oils of the irradiated variants which varies between 70.4-74.8% in Trabant and between 70.4 – 78.4% in Abacus, compared to the original hybrids Trabant-74.4% and Abacus-66.3%, respectively (Table 5).

Similar to the total content of these acids in the oil, there was a significant increase in the percentage of linoleic acid (C18:2) in M3-T1 hybrids of Trabant was 14.6% and M3-T15 was 17.6% compared to non-irradiated plants-7.8%.

The content of linoleic acid in the seeds of M3 A-10 and M3 generation of A-15 increased to 19.1%, compared to Abacus-17.2%. The variation in the fatty acid composition of the irradiated variants compared to the non-irradiated

Table 5. Fatty acid composition in seeds obtained from non-irradiated and M1, M2, and M3 segregating populations of Trabant and Abacus treated with 10 and 15 krad gamma rays

Fatty acids, %	Trabant	M1-T10	M1-T15	M2-T10	M2-T15	M3-T10	M3-T15
C12:0 Lauric acid	ND	0.1	0.2	0.1	0.1	0.1	0.2
C14:0 Myristic acid	0.1	0.2	0.2	0.2	0.2	0.1	0.1
C16:0 Palmitic acid	6.3	6.1	5.8	5.9	6.0	5.0	5.6
C18:0 Stearic acid	0.4	0.4	0.4	0.5	0.5	0.6	0.4
C18:1 Oleic acid	74.4	74.8	72.8	71.7	74.2	74.8	70.4
C18:2 Linolic acid	7.8	6.7	8.1	8.1	8.6	14.6	17.6
C18:3 Linoleic acid	1.1	1.5	1.5	2.0	3.4	4.6	5.5
Fatty acids, %	Abacus	M1-A10	M1-A15	M2-A10	M2-A15	M3-A10	M3-A15
C12:0 Lauric acid	ND	0.1	0.1	0.1	0.1	0.1	0.2
C14:0 Myristic acid	0.1	0.1	0.2	0.2	0.2	0.1	0.1
C16:0 Palmitic acid	7.5	6.7	6.3	6.5	6.7	5.9	5.7
C18:0 Stearic acid	2.3	1.8	2.0	1.9	1.2	0.3	0.3
C18:1 Oleic acid	66.3	69.3	67.0	67.8	68.5	67.5	73.0
C18:2 Linolic acid	17.2	15.5	17.4	17.2	18.3	19.1	15.9
C18:3 Linoleic acid	4.4	3.1	3.5	4.2	5.9	6.9	4.6

*ND – not detected

ones suggests variation in the genotypes in the decaying hybrid populations of Trabant and Abacus.

Linolenic acid was a very valuable acid, but it was easily oxidized, which reduces the quality of food products during storage. The oils with a high content of C18:3 are used for technical purposes and combustion. The tendency in the breading programs was to reduce the content of unwanted linolenic acid below 3%. From the results in Table 5, it was clear that the irradiated Abacus A10 and A15 in M1 plants have a reduced value of the fatty acid compared with the control ones. In contrast in M3 generation, an increase in the concentration of fatty acid was established.

NIRS analysis and quantitative analysis

Seeds of the selected genotypes of Trabant and Abacus as well as their M1, M2, and M3 generations were screened for quantitative and qualitative fatty acid composition using gas chromatography and NIRS. In oilseed rape, NIRS was useful for the estimation of oil, protein and glucosinolate contents as well as fatty acid composition simultaneously in a quick and non-destructive manner using a small number of seed samples (Velasco et al., 1997). Special adapters and separate NIRS calibrations have been developed to predict seed quality traits in those samples (Sato et al., 1998; Velasco and Becker, 1998; Velasco et al., 1999). According to Niewitetzki, 2010 the oil quality was determined by the genotype of the seed, a selection can be performed among single seeds of segregating populations were analyzed by NIRS and gas chromatography. It was found that the frequency of change in the amount of C18 fatty acids after gamma irradiation was not higher than 1 in 5000. Therefore, it was necessary to find a rapid and effective method for screening a decaying mutant population of rapeseed variants.

The obtained spectral data show that it was possible to obtain non-destructive spectra of rapeseed, even individual seeds (Supplementary figure 1). In the obtained spectra, differences were observed both between non-irradiated and irradiated seeds and between the obtained generation of irradiated seeds and between seeds of different varieties. The largest variations were observed around 1214 and 1430 nm. The absorption around 1214 nm was associated with CH-bonds of fats, and in the range, 1420 – 1440 nm mainly with OH, N-H bonds (Supplementary figure 1). Therefore, variations in the chemical composition of the seeds were reflected in their absorption spectrum in the studied spectral range. Developed equations for determining the number of fatty acids in the analysis of samples from the harvest 2018 – 2021 was done by PLS regression. The results obtained were presented in Table 6. The SEC parameter represents a standard calibration error that was related to an equation, and the SECV was a cross-check error, which was estimated as an error that occurs when analyzing unknown samples. Rcv and Rcal were multiple correlation coefficients in cross-checking and calibration. The RPDcv parameter was related to the standard deviation of the corresponding SECV cross-check error parameter. This result agreed with the results of other studies (Wan et al., 2018; Du et al., 2021; Eifler et al., 2021;).

The results show that there was a strong relationship between spectral data and fatty acid content. For most of them, the multiple correlation coefficients in cross-checking and calibration were greater than 0.9. The accuracy of determining the content of oleic, stearic and palmitic acid was the best. For them, the correlation coefficients during calibration and verification were greater than 0.93, and the RPDcv parameter was greater than 3, which shows very good accuracy of the determination. Accuracy sufficient for screening and distinguishing between low and high content samples were obtained for the determination of linolenic and eicosenic acid. Therefore, these equations can be used for non-destructive analysis and evaluation of the fatty acid content of rapeseed.

SIMCA models for classification of samples based on

Table 6. Statistical parameters of the equations for determining the amount of fatty acids in the analyzed samples from the 2021 harvest

Fatty acids	SEC	Rcal	SECV	Rcv	RPDcv
Saturated FA	0.43	0.986	0.18	0.932	2.78
Unsaturated FA	0.11	0.967	0.13	0.952	3.31
Palmitic acid	0.10	0.959	0.12	0.937	5.88
Stearic acid	0.08	0.967	0.10	0.943	3.04
Oleic acid	0.97	0.974	1.13	0.962	3.68
Linolic acid	0.90	0.938	1.44	0.810	1.67
Linoleic acid	0.25	0.964	0.37	0.905	2.36
Arachidonic acid	0.046	0.913	0.061	0.820	1.75
Eicosenoic acid	0.047	0.933	0.052	0.912	2.41

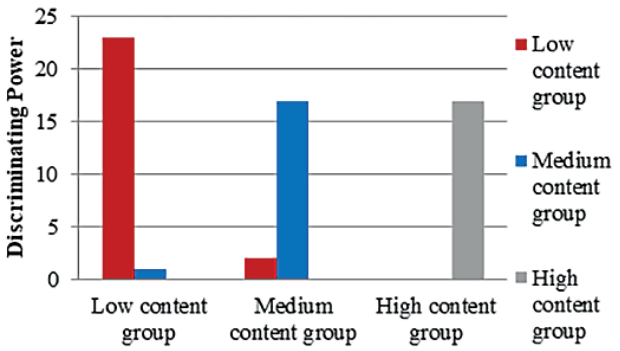

Fig. 2 A. Results of SIMCA models for classification of samples based on oleic acid content (18:1)

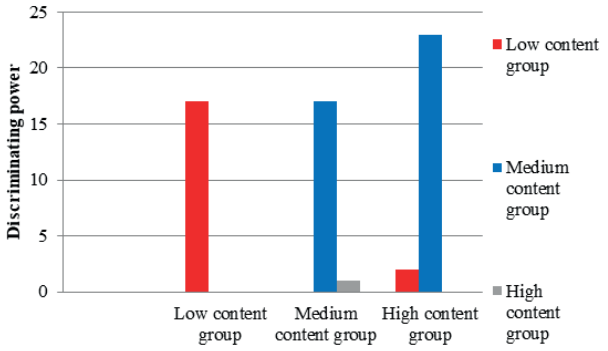

Fig. 3 A. Results from SIMCA models for classification of samples based on linoleic acid content (18:2)

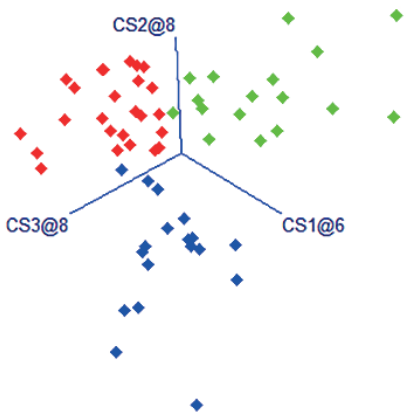

Fig. 2 B. Classification based on oleic acid content 18:1. Red – low content group, blue – medium content group, green – high content group

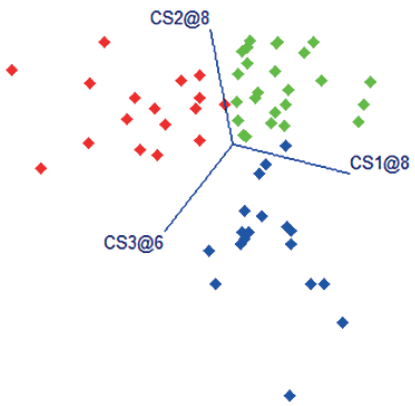

Fig. 3 B. Classification of samples based on linoleic acid content (18:2). Red – low content group, blue – medium content group, green – high content group

oleic acid content (18:1) and Principal Component Analysis were used to clarify the data structure. In it, the spectral data were transformed into new factors, which was a certain way to describe the information in the spectral data. The first factor describes the maximum of the spectral information, the second the maximum of the rest of the information, and so on. Thus, all spectral information about the samples can be described by several such factors (Principal components). Samples from the 2021 harvest were divided into 3 groups depending on the content of oleic (18:1) and linoleic acid (18:2) groups with low, medium, and high content (Figure 2 and 3). Based on the spectra of the samples, redeem the groups with developed SIMCA models for classification. Good accuracy was obtained in distinguishing the samples depending on the content of these fatty acids.

Conclusions

Irradiation with 10 and 15 krad gamma rays 60Co had a different effect on both genotypes. Trabant was improved in M3 generation as the plants were shorter with better branching and increase number of siliques per plant and seeds per silique. The seed weight per plant in the progeny of the variant with a higher irradiation dose was 90% bigger than the standard. The mutation procedure for Abacus leads to smaller changes – shortening of the plant stem, increased number of siliques per plant and seeds per silique. The higher coefficient of variability in M3 allows continuing the selection in the next progenies. The changes in the fatty acid composition of the individuals from M1, M2, and M3 generation of the individuals treated with physical mutagen were associated with changes in the biometric indica-

tors. Most of the studied plants from M1 to M3 generations of irradiated variants showed increased levels of linoleic and linolenic acids and lower levels of oleic acid compered to non-irradiated variants. The present study demonstrates the successful development calibrations for total oil and fatty acid profile in combination with biometric studies of the rapeseed mutants.

Author Contributions

Marina Marcheva – writing and editing the original draft, conceptualization, data calculation, plant experiments, software analyses and validation; Mariana Petkova – editing the original draft, conceptualization, molecular methodology, and resources; Stefka Atanassova – NIRS analysis, spectral measurements of the tested samples, supervision, conceptualization, and resources.

Acknowledgments

This article was completed with financial support from the Centre of Research, Technology Transfer, and Protection of Intellectual Property Rights of the Agricultural University – Plovdiv on project 10–17.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare that they have no identified competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: **September, 30, 2022**; *Approved*: **October, 25, 2022; *Published:*** **December, 2023**