Stanisław Spasibionek
Instytut Hodowli i Aklimatyzacji Roślin, Oddział w Poznaniu

Variability of fatty acid composition in seed oil of winter rapeseed (*Brassica napus* L.) developed through mutagenesis*

Zmiany składu kwasów tłuszczowych w nasionach rzepaku ozimego (*Brassica napus* L.) uzyskane na drodze mutagenezy

Key words: winter rapeseed *Brassica napus* L., chemical mutagenesis, fatty acids, oleic acid, linoleic acid, linolenic acid, oleic and linoleic desaturation ratio

Induced mutagenesis plays a significant role in the development of new fatty acid variability in seeds of oilseed crops. The aim of investigations was to find out optimal conditions for mutagenesis favourable to the increase of variability of polyunsaturated fatty acids in winter oilseed rape and to obtain mutated lines with high oleic and reduced linolenic acid content. Different conditions of mutagenesis with the use of ethyl methanesulphonate (EMS) were investigated. The mutation with the use of EMS has been performed on double low inbred line PN3756/93. After selection in several subsequent generations M_2 – M_7, two mutants M-10453 and M-10464 with significantly increased oleic acid content (over 75%) and one mutant M-681 with high linoleic and low linolenic acid content (respectively 27,5 and 2,7%) were selected. Five mutants M-1286/42, M-1288/27 M-1290/361, M-1292/59 and M-1292/271 obtained in different EMS treatment conditions performed on another double low line PN 5282/98 and selected in M_3 – M_6 generations are characterized by increased level of oleic acid average (74,6–77,1%) and reduced linolenic acid content average (4,0–4,8%). Significant changes obtained in the content of fatty acids in oil seeds suggest that activity levels of enzymes Δ12 and Δ15 desaturases which influence the content of oleic, linoleic and linolenic acids synthesis undergo considerable damages. It is the effect of mutations in genes *fad2 or fad3 (fatty acid desaturase).*

Słowa kluczowe: rzepak ozimy, *Brassica napus* L., mutageneza chemiczna, kwasy tłuszczowe, kwas oleinowy, kwas linolowy, kwas linolenowy, stopień desaturacji kwasu oleinowego i linolowego

Oleje roślinne, a zwłaszcza występujące w nich 18-węglowe kwasy tłuszczowe, mają istotne znaczenie w żywieniu człowieka, a także mogą być wykorzystane dla różnych celów technicznych. Stąd potrzebne są oleje o różnym składzie kwasów tłuszczowych. Znaczącą rolę w tworzeniu nowej zmienności kwasów tłuszczowych u roślin oleistych odgrywa indukowana mutageneza.

* Badania wykonano częściowo w ramach projektu badawczo-promotorskiego: 5 PO6A00819 "Wykorzystanie mutagenezy indukowanej chemicznie dla tworzenia nowych genotypów rzepaku ozimego o zmienionym składzie kwasów tłuszczowych".

Prace nad indukowaną mutagenezą rozpoczęto w 1993 roku wywołując mutację poprzez działanie na nasiona rzepaku ozimego 0,5 i 1% roztworami metanosulfonianu etylu (EMS) w czasie 2 godzin. Dla osiągnięcia istotnie większych zmian nasiona pokolenia M_2 wyselekcjonowanej linii 1207 poddano powtórnemu działaniu (EMS) o stężeniach 2, 5 i 8% przez okres 2 godzin. W wyniku tych prac znaleziono mutanty M-10453 i M-10464 o wysokiej zawartości kwasu oleinowego (odpowiednio 76,1 i 76,6%) przy jednoczesnym obniżeniu zawartości kwasu linolowego (8,7 i 8,8%) i kwasu linolenowego (7,2 i 7,4%), a także mutanta M-681 o wysokiej zawartości kwasu linolowego (27,5%) i znacznie obniżonej zawartości kwasu linolenowego (2,7%). Większość roślin mutantów była półkarłowa i karłowa. Niektóre rośliny mutanta M-681 charakteryzowały się również deformacjami rozety oraz pędu głównego (Spasibionek 2006). Aby uniknąć długoletnich prac selekcyjnych spowodowanych deformacjami morfologicznymi w 1998 roku zastosowano nowy, łagodniejszy sposób indukowania mutacji. Nasiona rodu PN 5282/98 poddano tylko jednokrotnemu działaniu roztworem EMS o stężeniach 0,5 i 1% wydłużając natomiast czas jego ekspozycji o 2 i 4 godziny w stosunku do czasu zastosowanego w pierwszym eksperymencie w roku 1993. Pozostałe warunki prowadzenia mutagenezy tj. wstępne moczenie nasion, przygotowanie mutagenu oraz czas wymywania mutagenu nie uległy zmianie.

Intensywne prace selekcyjne prowadzone w pokoleniach mutantów od M_3 do M_6 doprowadziły do znalezienia pięciu zmutowanych genotypów M-1286/42, M-1288/27 M-1290/361, M-1292/59 i M-1292/271 o zmienionym składzie kwasów tłuszczowych ale bez deformacji morfologicznych. W otrzymanych liniach mutantów wzrosła zawartość kwasu oleinowego średnio od 74,6 do 77,1%, oraz obniżyła się zawartość kwasu linolenowego średnio (od 4,0 do 4,8%). Uzyskane istotne zmiany w kierunku wysokiej zawartości kwasu oleinowego i obniżonej zawartości kwasu linolowego i linolenowego w oleju nasion badanych linii wskazuje na mutację genu lub genów *fad2* i *fad3* warunkujących aktywność desaturazy kwasów oleinowego i linolowego.

Introduction

Elimination of erucic acid increased the content of monounsaturated oleic acid and the sum of polyunsaturated acids: linoleic and linolenic. Majority of Polish and foreign double low winter oilseed rape cultivars provide oil, in which the share of acids with eighteen atoms of carbon mono- and polyunsaturated amounts to: oleic acid 60–65%, linoleic acid 18–22%, linolenic acid 8–11%, and the sum of saturated fatty acids content 7%.

This fatty acid composition makes rapeseed oil an universal oil: perfect for edible purposes (Scarth and McVetty 1999, Clifton 1999, Pedersen et al. 2000) and good for production of biofuel biocomponents.

In comparison to other plants oil the rapeseed oil exhibits a very low content of saturated fatty acids and relatively high content of essential fatty acids linoleic and α-linolenik. Rapeseed oil is the only plant oil characterized by an optimal proportion 2 : 1 of linoleic and α-linolenic acid. Good proportion of these two fatty acids plays a significant role in prevention of coronary heart disease (Zatoński et al. 2008).

High oleic acid content and moderate polyunsaturated fatty acid content allow to use this oil for frying as well as for production of biofuel biocomponents. Oil of double low varieties meets requirements of European Standard as a row material

for processing for methyl esters (RME) — biocomponents of biodiesel. However oil containing over 75% of oleic acid and not more than 3–5% of linolenic acid would be better for biodiesel components production and for frying (Scarth et al. 1999, Carré et al. 2007, Matthäus 2007). Especially the decrease of linolenic acid improves oxidation stability of oil and results in enhanced shelf life of oil.

Mutation induction is an effective way to enrich the genetic variability available in plant breeding (Micke et al. 1987). Many examples of successful mutation breeding confirm that induced mutants are effective and important instruments also in the case of oil plants (Röbbelen 1990, Velasco et al. 1999, Schierholt et al. 2001). Induced mutagenesis plays a significant part in the development of new fatty acid variability in oilseed crops. The first mutation experiment resulting in a substantial modification of fatty acid composition of crops was initiated in Germany in 1968 by Rakow. The author isolated rapeseed mutants having either reduced or increased linolenic acid content (Rakow 1973). More recently, Auld et al.(1992) in *B. napus* and *B. rapa*, as well as Wong and Swanson (1991), Rücker and Röbbelen (1995), Byczyńska et al. (1996) and Spasibionek (2006) in *B. napus*, developed several mutants with alternations in their content of oleic, linoleic and linolenic acid.

The aim of experiments was to find optimal conditions of mutagenesis to increase variability of polyunsaturated fatty acids in winter rapeseed and to obtain mutated lines of winter rapeseed with high oleic and reduced linolenic acid content maintaining performance of good agronomic traits.

Materials and methods

The first mutation experiment was performed in 1993. Seeds of the canola quality *Brassica napus* winter oilseed rape line PN 3756/93 bred in Oil Crop Department of Plant Breeding and Acclimatization Institute in Poznan, were used for mutagen treatment. This line is characterized by high seed oil content of 48.3%, and its fatty acid composition is typical of double low winter oilseed rape. The fatty acid composition of the seed oil was: palmitic ($C_{16:0}$) 4,5%, stearic ($C_{18:0}$) 1,1%, oleic ($C_{18:1}$) 64,1%, linoleic ($C_{18:2}$) 18,2%, linolenic ($C_{18:3}$) 10,4% and eicosenoic ($C_{20:1}$) 1,1%. This line had also a very low glucosinolate content of 5,2 µmol/g of seeds.

The alkylating substance — ethyl methanesulphonate (EMS) was used as mutagen. Seeds were initially soaked in distilled water at 2°C for twelve hours. Then the seed surface was blotted from water with filter paper and seeds were treated with 0,5 or 1,0% solutions of EMS. The EMS solutions in phosphate buffer (pH about 7) were prepared just before use. Seeds were left in the mutagen solution for 2 hours at 4°C followed by 2 hours at room temperature (about 23°C). Then seeds were washed for 16 hours with running tap water to remove EMS completely (Table 1).

Table 1

Conditions of mutagenesis induction (1993 year) — *Warunki prowadzenia mutagenezy*

Method *Metoda*	Presoaking [temp./time] *Wstępne moczenie nasion [temp./czas]*	Mutagen concentration *Stężenie mutagenu* [% v/v]	Exposition [temp./time] *Ekspozycja [temp./czas]*	Time et washing out of mutagen *Czas wymywania mutagenu*
PN 3756/93 — strain — *ród wyjściowy*				
I	2^oC/12 h	0,5	two-stage *dwustopniowa* 4^oC/2 h; 23^oC/2 h	tap water for 16 h *bieżąca woda przez 16 godz.*
II		1,0		

After surface blotting, seeds were planted directly into soil in pots. The M_1 generation was grown in 1993/1994. Chemical analyses were performed on single M_2 seeds, and the fatty acid modified line 1207/94 was selected in 1994.

Twenty nine plants were selected from line 1207/94 in 1995. Seeds of the M_2 generation collected from these plants were treated again in 1996 using EMS concentrations of 2,0; 5,0 and 8,0%. The second treatment was applied expecting that it may increase the frequency of mutations and cause greater genetic changes. The seeds for the second treatment were characterized by an increased oleic acid content (average 70,3%), reduced polyunsaturated acid content (average linoleic of 15,1% and linolenic 6,7%). Preliminary soaking of seeds, preparation of EMS solutions, temperatures, duration of seed treatment and EMS washout were the same as in the first experiment (Table 2).

Table 2

Conditions of mutagenesis induction (1996 year) — *Warunki prowadzenia mutagenezy*

Method *Metoda*	Presoaking [temp./time] *Wstępne moczenie nasion [temp./czas]*	Mutagen concentration *Stężenie mutagenu* [% v/v]	Exposition [temp./time] *Ekspozycja [temp./czas]*	Time et washing out of mutagen *Czas wymywania mutagenu*
29 plants of M_2 generation selected from line 1207/94 *Mieszanina 29 pojedynków M_2 z linii PN 1207/94*				
I	4^oC/12 h	2,0	two-stage *dwustopniowa* 4^oC/2 h; 20^oC/2 h	tap water for 16 h *bieżąca woda przez 16 godz.*
II		5,0		
III		8,0		

The application in 1996 of high mutagen concentrations as well as multiple repetition of mutagenesis caused not only large changes in contents of 18 carbon acids in oil of seeds, however it was the cause of unfavourable morphological deformations and reduced vitality of plants.

In growing season 1998/99, the seeds of double low winter oilseed rape strain PN 5282/98 with typical fatty acid composition in oil: 4,7% of palmitic acid, 1,5% of stearic acid, 67,1% of oleic acid, 16,8% of linoleic acid and 8,6% of linolenic acid were treated with EMS.

In order to develop new mutative changes of fatty acids composition, lower concentration of EMS (0,5% and 1%) was used and the time of seed exposition to mutagen was longer than in methods I – V (Table 2 and 3).

Table 3

Conditions of mutagenesis induction (1998 year) — *Warunki prowadzenia mutagenezy*

Method *Metoda*	Presoaking [temp./time] *Wstępne moczenie nasion [temp./czas]*	Mutagen concentration *Stężenie mutagenu* [% v/v]	Exposition [temp./time] *Ekspozycja [temp./czas]*	Time et washing out of mutagen *Czas wymywania mutagenu*
PN 5282/98 — strain — *ród wyjściowy*				
VI	20°C/12 h	0,5	two-stage *dwustopniowa* 4°C/2 h; 23°C/4 h	tap water for 16 h *bieżąca woda przez 16 godz.*
VII			two-stage *dwustopniowa* 4°C/2 h; 23°C/6 h	
VIII		1,0	two-stage *dwustopniowa* 4°C/2 h; 23°C/4 h	
IX			two-stage *dwustopniowa* 4°C/2 h; 23°C/6 h	

The next most labour consuming stage was the search for plants with modified fatty acid compositions. These plants formed the basis of new lines with improved fatty acid compositions which were stabilized through continued inbreeding connected with fatty acid selections conducted during several generations.

Seeds of the M_2 generation collected from M_1 plants were individually screened using a test for linolenic acid content (McGregor 1974, Byczyńska at al. 1994). This test was conducted on spots of oil pressed to filter paper from individual seeds. Reaction of linolenic acid with thiobarbituric acid was used to

develop colour. Seeds giving lighter spots were analysed using gas chromatography to verify level of linolenic acid content (Byczyńska and Krzymański 1969).

Selections in later generations were conducted with the use of the half seed method. Individual seeds soaked in water over night were used to prepare embryos. One cotyledon with the rootlet was placed in a peat cork, and the second cotyledon was used for examination of fatty acid compositions. Only plants from embryos with changed contents of mono — or polyunsaturated acids were grown. After vernalization, some plants were grown in the greenhouse and others were transplanted directly into field plots and observed under natural field conditions.

The statistical analysis of qualitative and quantitative data of successive generations of mutants was performed using Excel program. The selection progress in subsequent generations is illustrated in tables and histograms. Calculations were also made for oleic desaturation ratio (ODR) and linoleic desaturation ratio (LDR). These indices were calculated according to formulas given by Pleines and Friedt (1988):

$$\mathrm{ODR} = \frac{\%C_{18:2} + \%C_{18:3}}{\%C_{18:1} + \%C_{18:2} + \%C_{18:3}} \times 100$$

$$\mathrm{LDR} = \frac{\%C_{18:3}}{\%C_{18:2} + \%C_{18:3}} \times 100$$

where:
$C_{18:1}$ — oleic acid — *kwas oleinowy* $C_{18:2}$ — linoleic acid — *kwas linolowy*
$C_{18:3}$ — linolenic acid — *kwas linolenowy*

In order to compare the distributions of fatty acid content among following generations of mutants it was necessary to make corrections for their fluctuation over years. Annual fluctuations observed in fatty acid composition of the check lines PN 3756/93 and PN 5282/98 were used for correction of fatty acid compositions of mutants. Corrections were done in an additive manner.

Results and discussion

Very large populations of plants or seeds were investigated, especially in segregating M_2 generations, because the probability of finding desirable mutants is very small. Rakow (1973) analyzed about 15 000 single seeds in the M_2 generation to find two mutants M-57 and M-364 with changed linoleic and linolenic acid contents. Similarly, Auld et al. (1992) examined a large population of 39 504 individual seeds of the M_2 generation to find the mutant X-82. The first step to find our three mutants M-10453, M-10464 and M-681 was the examination of

individual seeds of the M_2 generation collected from 21 480 plants. The screening for linolenic acid content of a huge number of M_2 generation seeds was done with the thiobarbituric test. A population of 1 339 M_2 seeds with reduced linolenic acid content was selected. After verification with gas chromatography, only 29 M_2 seeds were chosen and grown into plants. The line 1207/94, selected from these plants, had a significantly changed fatty acid composition. The content of oleic acid increased to 70,3%, but linoleic acid content decreased to 15,1% and linolenic acid to 6,7%. These changes were significant in relation to the fatty acid composition of the original line PN 3756/93 in the same year. The chosen line had also high seed oil content (average 49.3%).

The seeds of line 1207/94 were treated again with EMS. Selection and inbreeding were conducted during several subsequent years. Altogether 6 593 plants were analyzed and examined: for $(M_2)_2$ — 302 plants, for $(M_2)_3$ — 1 143 plants, for $(M_2)_4$ — 2 872 plants, for $(M_2)_5$ — 694 plants, for $(M_2)_6$ — 1 225 plants and for $(M_2)_7$ — 357 plants (Table 4).

Table 4

Scheme of M-10453, M-10464 and M-681 mutants selection
Schemat selekcji mutantów M-10453, M-10464, M-681

Number of plants *Liczba roślin*	First EMS treatment *I traktowanie EMS* PN 3756/93	Second EMS treatmeent *II traktowanie EMS* PN 1207/94	Selection in generations M_2 *Selekcja w pokoleniu M_2*					
	M_1	M_2	$(M_2)_2$	$(M_2)_3$	$(M_2)_4$	$(M_2)_5$	$(M_2)_6$	$(M_2)_7$
Number of selected individual plants *Liczba selekcjonowanych roślin*	21480	1339	302	1143	2872	694	1225	357
Number of chosen individual plants *Liczba wybranych roślin*	1339	29	302	3	8	32	55	104

As a result, the following mutants were found and stabilized: two mutants, M-10453 and M-10464, with high oleic acid content and decreased linoleic and linolenic acid contents, and mutant M-681 with high linoleic acid content and considerably reduced linolenic acid content (Table 5).

Table 5

Comparison of quality and quantity traits of the PN 3756/93 strain and M-10453, M-10464, M-681 mutants investigated in field trials — *Porównanie cech jakościowych i ilościowych rodu wyjściowego PN 3756/93 z cechami mutantów M-10453, M-10464, M-681 badanych w doświadczeniach polowych*

Trait — Cecha	PN 3756/93	M-10453	M-10464	M-681
$C_{16:0}$ – palmitic acid [%] *kwas palmitynowy*	5,0	4,6 [± 0,6]	3,9 [± 1,2]	4,7 [± 1,0]
$C_{18:0}$ – stearic acid [%] *kwas stearynowy*	1,2	1,2 [± 0,4]	1,2 [± 0,3]	1,8** [± 0,5]
$C_{18:1}$ – oleic acid [%] *kwas oleinowy*	65,0	76,1** [± 3,8]	76,6** [± 3,2]	61,0 [± 5,6]
$C_{18:2}$ – linoleic acid [%] *kwas linolowy*	18,4	8,7** [± 2,0]	8,8** [± 2,4]	27,5** [± 4,2]
$C_{18:3}$ – linolenic acid [%] *kwas linolenowy*	8,7	7,2** [± 1,4]	7,4** [± 1,0]	2,7** [± 1,2]
ODR oleic desaturation ratio *stopień desaturacji kwasu oleinowego*	29,4	17,3** [± 3,4]	17,4** [± 2,8]	33,2** [± 5,5]
LDR linoleic desaturation ratio *stopień desaturacji kwasu linolowego*	32,1	45,5** [± 4,1]	45,8** [± 6,1]	9,1** [± 3,8]
Fat content [%] *Zawartość tłuszczu*	50,8	48,4** ± 0,8	47,7** ± 0,5	46,6** ± 1,3
Glucosinolates [μM/g seeds] *Glukozynolany*	7,2	12,2** ± 0,7	8,7 ± 1,0	10,7** ± 1,9

* significant difference in comparison with PN 3756/93 at the α level ≤ 0,05; ** ≤ 0,01
** istotność w porównaniu z rodem PN 3756/93 na poziomie α ≤ 0,05; ** ≤ 0,01*

Oleic acid content in seed oil of M-10453 and M-10464 mutants significantly increased and was stabilized at the level of 76,1 and 76,6%, respectively; linoleic and linolenic acid content decreased to the value of 8,7 and 8,8% respectively and linolenic acid content decreased to the value of 7,2 and 7,4% respectively, in comparison with the strain PN 3756/93, that had 65% oleic acid content, 18,4% linoleic acid content and 8,7% linolenic acid content. These big changes of fatty acid composition confirm significantly changed values of oleic desaturation ratio (ODR) at the level of 17,3 and 17,4 and changed values of linoleic desaturation ratio (LDR) at the level of 45,5 and 45,8 of mutant M-10453 and of mutant M-10464, respectively. In compared strain PN 3756/93 value of ODR amounted to 29,4 and LDR amounted to 32,1.

Obtained modification of fatty acid composition in direction of high oleic acid content and decreased linoleic and linolenic acid content of seed oil of both mutants M-10453 and M-10464 proved that probably the gene responsible for desaturation of oleic acid was mutated.

Selection of mutant M-681 was very difficult due to more drastic changes induced by mutagen treatment. This caused large changes in contents of C_{18}-carbon acids but also small vitality and large morphological deformations of plants. In such a situation, selections should be conducted on large populations (Auerbach 1976) to increase the probability of finding the desired mutants not only with regard to fatty acids but also with good vigor and with less side effects which are difficult to remove. The intensive selection in $(M_2)_4 – (M_2)_7$ generations stabilized reduced linolenic acid content as well as the increased linoleic acid content. A considerably larger problem during selection was to obtain lines with good agronomic parameters.

In the mutant M-681 high level of linoleic acid content up to 27,5% and very decreased level of linolenic acid content to 2,7% were obtained. Important changes, especially of linolenic acid content confirm significant changes of LDR value to the level of 9,1. In the strain PN 3756/93 the value of LDR amounted to 32,1.

Fat content in seeds of mutants was lower than in the PN 3756/93 strain (50,8%) and amounted to 48,4% for mutant M-10453, 47,7% for mutant M-10464, 46,6% for mutant M-681. Glucosinolate content in seeds of mutant M-10453 amounted to 12,2 μM/g of seeds and was higher in comparison with the PN 3756/93 strain (7,2 μM/g of seeds). Glucosinolate content in seeds of the second M-10464 mutant was lower than in the mutants M-10453 and M-681 (10,7 μM/g of seeds) and amounted to 8,7 μM/g of seeds (Data partly published in Spasibionek 2006).

The first step to find our five mutants M-1286/42, M-1288/27 M-1290/361, M-1292/59 and M-1292/271 was the examination of individual seeds of the M_2 generation collected from 12 000 plants (Table 6).

After treatments, individual seed and plant selections were made for changes in fatty acid composition during several generations of inbreeding. Self-pollinated plants with changed fatty acid compositions were inbred to obtain genetically homozygous and stable mutant lines. A population of 1 494 M_2 seeds was selected to increase oleic and reduce linolenic acid content with gas chromatography. After verification 107 plants of M_2 generation were obtained, in which oleic acid content increased up to 72,8%, and linolenic acid content decreased to 5,4%.

The application of low mutagen concentrations as well as longer time of exposition to mutagen caused large changes in the contents of 18 carbon fatty acids in oil of seeds.

Table 6

Scheme of M-1286/42, M-1288/27 M-1290/361, M-1292/59 and M-1292/271 mutants selection — *Schemat selekcji mutantów M-1286/42, M-1288/27 M-1290/361, M-1292/59, M-1292/271*

Number of plants *Liczba roślin*	EMS treatment T*raktowanie EMS* PN 5282/98	Selection in generations M_2 *Selekcja w pokoleniu* M_2				
	M_1	M_2	M_3	M_4	M_5	M_6
Number of selected individual plants *Liczba selekcjonowanych roślin*	12000	1494	876	225	77	194
Number of chosen individual plants *Liczba wybranych roślin*	1494	107	61	25	30	101

In $M_3 – M_6$ generations five mutated lines with increased level of oleic acid average from 74,6 to 77,1% and reduced linolenic acid content average from 4,0 to 4,8% were selected (Table 7). Beside obtained significant changes in fatty acids composition, the mild conditions of mutagen treatment did not cause morphological deformations, hence the mutants kept good vigour.

These big changes of fatty acid composition confirm significantly changed values of oleic desaturation ratio (ODR) at the level approximately from 16,7 to 19,4 changed values of linoleic desaturation ratio (LDR), at the level approximately from 23,7 to 31,0 of five mutants M-1286/42, M-1288/27 M-1290/361, M-1288/27 and M-1292/271. In compared strain PN 5282/98 value of ODR amounted to 27,5 and LDR amounted to 33,9. Significant changes obtained in the content of fatty acids in oil seeds suggest that activity levels of enzymes Δ12 and Δ15 desaturases which influence the content of oleic, linoleic and linolenic acids synthesis undergo considerable damages. It is the effect of mutations in genes *fad2 or fad3 (fatty acid desaturase)*.

Fat content in seeds of mutants was lower than in the PN 5282/98 strain (48,7%) and amounted to 45,7–46,8%. Glucosinolate content in seeds of five mutants amounted to 6,3–9,1 μM/g of seeds and was lower in comparison with the PN 5282/98 strain (10,4 μM/g of seeds) (Table 7).

Polyunsaturated fatty acid content of rapeseed oil is intermediate among the vegetable oils, lower than corn oil, soybean oil and sunflower oil, and higher than olive or palm oil. Linolenic acid is recognized as an essential fatty acid and has a role in reducing plasma cholesterol levels (Eskin et al. 1996). The ratio (2 : 1) between

Table 7

Changes of oleic, linoleic and linolenic acid content in the seeds of mutants selected in comparison with original strain PN 5282/98
Zmiany zawartości kwasów oleinowego, linolowego i linolenowego w nasionach wyselekcjonowanych mutantów w porównaniu z rodem wyjściowym PN 5282/98

Trait — *Cecha*	PN 5282/98	M-1286/42	M-1288/27	M-1290/361	M-1282/59	M-1292/271
$C_{16:0}$ palmitic acid [%] *kwas palmitynowy*	4,7	4,7 (±06)	4,3 (±0,3)	4,4* (±0,2)	4,2** (±0,4)	4,4* (±0,4)
$C_{18:0}$ stearic acid [%] *kwas stearynowy*	1,5	2,0** (±0,4)	2,0** (±0,3)	1,9* (±0,2)	2,2** (±0,7)	2,3** (±0,4)
$C_{18:1}$ oleic acid [%] *kwas oleinowy*	67,1	74,6** (±3,1)	74,7** (±1,4)	75,3** (±3,0)	77,1** (±4,1)	76,4** (±3,0)
$C_{18:2}$ linoleic acid [%] *kwas linolowy*	16,8	13,6* (±3,8)	13,2* (±1,2)	12,9* (±2,6)	10,7** (±1,8)	11,7** (±2,1)
$C_{18:3}$ linolenic acid [%] *kwas linolenowy*	8,6	4,1** (±2,4)	4,8** (±0,9)	4,5** (±0,8)	4,8** (±1,0)	4,0** (±1,2)
ODR oleic desaturation ratio *stopień desaturacji kwasu oleinowego*	27,5	19,2** (±2,8)	19,4** (±1,5)	18,8** (±3,4)	16,7** (±2,7)	17,0** (±3,3)
LDR linoleic desaturation ratio *stopień desaturacji kwasu linolowego*	33,9	23,7** (±11,2)	26,5** (±2,8)	25,9** (±2,6)	31,0** (±2,6)	25,3** (±3,4)
Fat content [%] *Zawartość tłuszczu*	48,7	46,2** (±3,3)	46,4** (±3,3)	46,8* (±1,9)	46,3** (±2,8)	45,7** (±4,4)
Glucosinolates [µM/g seeds] *Glukozynolany*	10,4	7,8* (±2,2)	6,8** (±1,6)	8,2* (±1,7)	9,1 (±3,8)	6,3** (±4,2)

* significant difference in comparison with PN 5282/98 at the α level ≤ 0,05; ** ≤ 0,01
** istotność w porównaniu z rodem PN 5282/98 na poziomie α ≤ 0.05; ** ≤ 0.01*

linoleic (average 10,7%) and linolenic acid (average 4,8%) in new mutant oil M-1282/59 is also regarded as nutritionally favorable. However, in applications which require stability, vegetable oils which are high in polyunsaturated fatty acids such as rapeseed are stabilized using hydrogenation, with the resulting formation of trans fatty acids. The current recommendation from nutritionists is that the current levels of trans fatty acid in the diet should not be increased (Fitzpatrick and Scarth 1998).

Low linolenic oils in the first mutant M-681 and new five mutants M-1286/42, M-1288/27 M-1290/361, M-1292/59 and M-1292/271 were developed to increase the stability of rapeseed oil, reducing the requirement for hydrogenation. Low linolenic rapeseed oil demonstrated improved stability under conditions of accelerated storage with no changes in overall odor intensity or pleasantness. There were also significantly lower levels of free fatty acids during frying with low linolenic rapeseed oil with better flavour quality of frying products (Eskin et al. 1996).

Conslusions

Chemical mutagenesis is an effective way for development of new fatty acid variability in winter oilseed rape.

Changes in fatty acid composition observed in mutants suggest that EMS treatment frequently induces mutagenic changes which reduce activity of desaturase of oleic acid system.

Significant changes obtained in the content of fatty acids in oil seeds suggest that activity levels of enzymes Δ12 and Δ15 desaturases which influence the content of oleic, linoleic and linolenic acids synthesis undergo considerable damages. It is the effect of mutations in genes *fad2 or fad3*.

Selected mutant lines containing about 80 per cent of oleic acid and decreased polyunsaturated fatty acids in seed oil as well as developed molecular markers (Mikołajczyk et al. 2007) will be applied in breeding of high oleic and low linolenic cultivars of oilseed rape.

References

Auerbach C. 1976. Mutation Research. Problems, results and perspectives. Chapman and Hall, London: 504.

Byczyńska B., Krzymański J. 1969. Szybki sposób otrzymywania estrów metylowych kwasów tłuszczowych do analizy metodą chromatografii gazowej. / Quick method to obtain fatty acid methyl esther for gas chromatography analysis. (in Polish language). Tłuszcze Jadalne, XIII: 108-114.

Byczyńska B., Spasibionek S., Krzymański J. 1994. Selekcja pojedynczych nasion rzepaku ozimego na zawartość kwasu linolenowego. / Screening of individual seeds of winter oilseed rape for linolenic acid content. (in Polish language). Rośliny Oleiste – Oilseed Crops, XV: 87-90.

Byczyńska B., Krzymański J., Spasibionek S. 1996. Zmniejszenie zawartości kwasów wielonienasyconych w oleju rzepakowym w wyniku mutagenezy chemicznej / Decrease of polyunsaturated fatty acids content in rapeseed oil as result of chemical mutagenesis. Rośliny Oleiste – Oilseed Crops, XVII (1): 127-135.

Carré P., Evrard J., Judde A., Labalette F., Mazette S. 2007. Technological performances of low linolenic / high oleic rapeseed oils for food and non-food application. 12th International Rapeseed Congress Wuhan-China 5: 152-159.

Clifton P.M. 1999. Rapeseed: Nutritional aspects of a novel oil. 10th International Rapeseed Congress, Canberra-Australia 1999, CD-rom.

Eskin N.A.M., Macdonald B.E., Przybylski R., Malcomson L.J., Scarth R., Mag T., Ward K., Adolphe D. 1996. Canola Oil. Chapter 1 in Bailey's Industrial Oil and Fat Products. Fifth edition. Vol. 2. Edible oil and Fat Products: Oils and Oilseeds. John Wiley & Sons, Inc. N.Y.: 1-95.

Fitzpatrick K., Scarth R. 1998. Improving the health and nutritional value of seed oils. PBI Bulletin. NRC-CRC. January: 15-19.

Matthäus B. 2007. High oleic low linolenic rapeseed oil as alternative to common used frying oils. 12th International Rapeseed Congress Wuhan-China 5: 165-167.

McGregor D.I. 1974. A rapid and sensitive spot test for linolenic acid levels in rapeseed. Can. J. Plant Sci., 54: 213-214.

Micke A., Donini B., Maluszynski M. 1987. Induced mutations for crop improvement – a review. Trop. Agric. (Trinidad), 64: 259-278.

Mikołajczyk K., Dabert M., Karłowski W.M., Spasibionek S., Cegielska-Taras T., Bartkowiak-Broda I. 2007. Development of allele-specific SNP markers for the new low-linolenic mutant of winter oilseed rape. W: "Proceedings of the 12th International Rapeseed Congress, Wuhan, China, 2, 282-284.

Pedersen A., Baumstark M. W., Marckmann P., Gylling H., Sandström B. 2000. An olive oil-rich diet results in higher concentrations of LDL cholesterol and a higher of LDL subfractin particles than rapeseed oil and sunflower oil diets. Journal of Lipid Research, 41: 1901-1911.

Pleines S., Friedt W. 1988. Breeding for improved C_{18}-fatty acid composition in rapeseed (*Brassica napus* L.). Fat Sci. Technol., 90, 5: 167-171.

Rakow G. 1973. Selektion auf Linol- und Linolensäuregehalt in Rapssamen nach mutagener Behandlung. Z. Pflanzenzüchtung, 69: 62-82.

Röbbelen G. 1990. Mutation breeding for quality improvement. A case study for oilseed crops. Mutation Breeding Review, 6: 1-44.

Rücker B., Röbbelen G. 1995. Development of High Oleic Acid Rapeseed. Proc. 9th International Rapeseed Congress, Cambridge, 2: 389-391.

Scarth R., Mcvetty P.B.E. 1999. Designer oil canola a review of new food-grade *Brassica* oils with focus on high oleic, low linolenic types. Proc. 10th Int. Rapeseed Congress, Canberra, http://www.regional.org.au/au/gcirc/4/57.htm.

Schierholt A., Rücker B., Becker H.C. 2001. Inheritance of high oleic acid Mutations in winter oilseed rape (*Brassica napus* L.) Crop Sci., 41: 1444-1449.

Spasibionek S. 2006. New mutants of winter rapeseed (*Brassica napus* L.) with changed fatty acid composition. Plant Breeding, 125: 259-267.

Velasco L., Perez-Vich B., Fernandez-Martinez J.M. 1999. The role of mutagenesis in the modification of the fatty acid profile of oilseed crops. J. Appl. Genet., 40 (3): 185-209.

Wong R.S.C., Swanson E. 1991. Genetic modification of canola oil: high oleic acid canola. In: Fat and Cholesterol Reduced Foods: Technologies and Strategies (C. Haberstrohn, C.F. Morris eds.). Portfolio Publ. Co., USA: 153-164.

Zatonski W., Campos H., Willett W. 2008. Rapid declines in coronary heart disease mortality in Eastern Europe are associated with increased consumption of oils rich in alpha-linolenic acid. Eur. J. Epidemiol, 23: 3-10.