Mutants of *Brassica napus* with changed fatty acid composition

Stanisław Spasibionek, Jan Krzymański, Iwona Bartkowiak-Broda
Plant Breeding and Acclimatization Institute, Department of Oilseed Crops,
Strzeszynska 36, 60-479 Poznan, Poland, e-mail: sspas@nico.ihar.poznan.pl

ABSTRACT

Rapeseed oil with about 61% oleic acid, 20% linoleic acid, and 11% linolenic acid is at present accepted as the best oil for human nutrition. Relatively high level of polyunsaturated fatty acids accelerates the oxidation and reduces stability of oil. Oil quality can be improved by developing varieties with reduced polyunsaturated fatty acids and increased oleic acid content. Rapeseed oil with higher oleic acid content is not only better for edible purposes but also creates new possibilities for oleochemicals uses. Chemical mutagenesis was used in order to obtain changes in fatty acid composition. The seeds of double low winter oilseed rape strain with typical fatty acid composition in oil: 64,1 per cent of oleic, 18,2 per cent of linoleic and 10,4 per cent of linolenic acid were treated with ethyl methanesulphonate (EMS). Second treatment was performed on seeds of line PN 1207/94 selected from the M_2 generation. The M_2 generation of these populations was screened to identify mutants with low levels of polyunsaturated fatty acids using a modified thiobarbituric acid procedure. Fatty acid composition of seeds from the M_2 generation was estimated by gas chromatography method. In successive generations after mutagen treatment $(M_2)_2$-$(M_2)_7$, three mutants with changed fatty acid composition were selected. Mutant M-10453 has an increased oleic acid content to 76,1 per cent and decreased content of linoleic acid to 8,7 per cent and linolenic acid to 7,2 per cent. Mutant M-10464 has an increased oleic acid content to 76,6 per cent and decreased content of linoleic acid to 8,8 per cent and linolenic acid to 7,4 per cent. Mutant M-681 has an increased linoleic acid content to 27,5 per cent and decreased linolenic acid content to 2,7 per cent.

Key words: winter rapeseed – fatty acids – mutagenesis

INTRODUCTION

Vegetable oils, especially C18 fatty acids, which occur in them have essential importance for human nourishment and also they can be used for different technical purposes. Therefore, oils with different fatty acid composition are needed.

Existing double low winter oilseed rape cultivars produce oil with about 61% oleic acid, 20% linoleic acid and 11% linolenic acid.

The relatively high level of polyunsaturated fatty acids accelerates the oxidation and reduces stability of oil. Oil quality can be improved by developing varieties with reduced polyunsaturated fatty acids and increased oleic acid content. Rapeseed oil with 75% oleic acid content and with 4% linolenic acid content not only improves edible oil but also opens new possibilities for oleochemicals uses (Scarth & McVetty 1999).

Induced mutagenesis plays a significant part in the development of new fatty acid variability in oilseed crops (Spasibionek *et al.* 1999; Velasco *et al.* 1999). Following treatment of rapeseed with ethyl metanosulphonate, Rakow (1973) reported the induction of a mutant (M 57) that reduced linolenic acid content from 9,8% to 5,5%. Subsequent remutation of this line resulted in selections having as little as 3,2% linolenic acid (Röbbelen & Nitsch 1975). More recently, Rücker & Röbbelen (1995) in *Brassica napus*, obtained mutants with 75-80% oleic acid content in comparison to 61% in winter rapeseed cultivar Wotan. High oleic acid levels correspond to reduced polyunsaturated fatty acids to less than 10%.

This paper will describe the results of selection for stabilizing the traits of increased content of oleic acid and decreased contents of linoleic and linolenic acids in the oil of the mutants M-10453, M-10464, M-681 of double low winter oilseed rape.

MATERIALS AND METHODS

The object for mutagen treatment was the strain of winter double low oilseed rape PN 3756/93 with typical fatty acid composition in oil: 64,1% of oleic, 18,2% of linoleic and 10,4% of linolenic acid. The seeds of rapeseed were treated with 1% solution of ethyl metanosulphonate (EMS). Besides the single treatments the seeds of line PN 1207/94 M_2 generation with changed polyunsaturated fatty acids content were again treated with 2, 5, 8% solution of EMS.

First screening for polyunsaturated fatty acids was done by the use of thiobarbituric acid method to estimate the level of linolenic acid. This method can be applied for screening the large number of seeds in M_2 generation after treatment with mutagen. Fatty acids composition of seeds was estimated by gas chromatography.

RESULTS AND DISCUSSION

Frequency of occurance of desirable mutants is very low and it was necessary to investigate very big plant population. As a result of this research in $(M_2)_2$ generation two mutant plants with high oleic acid content and decreased linoleic and linolenic acid content and one mutant plant with increased linoleic acid and significantly decreased linolenic acid were found.

Inbreding performed to $(M_2)_7$ generation stabilized mutants with respect to changed fatty acid composition and morphology features (Table 1).

Table 1. Comparison of quality and quantity traits of the PN 3756/93 strain and M-10453, M-10464, M-681 mutants investigated in field trials.

Trait	PN 3756/93	M-10453	M-10464	M-681
Seeds				
$C_{16:0}$ – palmitic acid [%]	5,0	4,6 [± 0,6]	3,9 [± 1,2]	4,7 [± 1,0]
$C_{18:0}$ – stearic acid [%]	1,2	1,2 [± 0,4]	1,2 [± 0,3]	1,8** [± 0,5]
$C_{18:1}$ – oleic acid [%]	65,0	76,1** [± 3,8]	76,6** [± 3,2]	61,0 [± 5,6]
$C_{18:2}$ – linoleic acid [%]	18,4	8,7** [± 2,0]	8,8** [± 2,4]	27,5** [± 4,2]
$C_{18:3}$ – linolenic acid [%]	8,7	7,2** [± 1,4]	7,4** [± 1,0]	2,7** [± 1,2]
ODR – oleic desaturation ratio	29,4	17,3** [± 3,4]	17,4** [± 2,8]	33,2** [± 5,5]
LDR – linoleic desaturation ratio	32,1	45,5** [± 4,1]	45,8** [± 6,1]	9,1** [± 3,8]
Fat content [%]	50,8	48,4** ± 0,8	47,7** ± 0,5	46,6** ± 1,3
Glucosinolates [µM/g seeds]	7,2	12,2** ± 0,7	8,7 ± 1,0	10,7** ± 1,9
Plants				
Chlorophyll content in leaves – autumn	534,0	478,1*± 19,2	541,6 ± 21,7	529,7 ± 128,5
Chlorophyll content in leaves – spring	672,0	608,9 ± 23,6	638,7 ± 29,2	613,3 ± 75,4
Beginning of flowering [days from 1 January]	126	129** ± 1	129** ± 1	123** ± 1
Plant height [cm]	146,0	140,5 ± 2,9	124,1** ± 2,8	104,1** ± 21,6
Yield [dt/ha]	39,8	24,7** ± 3,4	18,6** ± 1,3	12,0** ± 8,0

[±] extreme values
significant difference in comparison with PN 3756/93 * at the α level ≤ 0,05; ** at the α level ≤ 0,01

Oleic acid content in seed oil of M-10453 and M-10464 mutants significantly increased and 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 in direction of high oleic acid content, 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.

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

Obtained changes of high linoleic acid content and significantly decreased linolenic acid content of seed oil in mutant M-681 proved that probably the gene responsible for desaturation of linoleic acid was mutated.

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.

During vegetation estimation of morphological traits of mutant lines was performed. In autumn and spring chlorophyll content in leaves was measured. In autumn and in spring the lowest chlorophyll content in leaves had mutant M-10453 (478,1 and 608,9 SPAD value - Soil Plant Analysis Development). Leaves of this mutant were light green. Chlorophyll content of the strain PN 3756/93 in leaves in autumn amounted to 534,0 and in spring amounted to 672,0.

The mutants had different beginning of flowering. The lines of M-10453 and M-10464 mutants began flowering 3 days later than the PN 3756/93 strain. The lines of M-681 mutant began flowering 3 days early than the PN 3756/93 strain. The lines of M-681 mutant were the lowest (104,1 cm).

None of mutants achieved seed yield level of the PN 3756/93 strain (39,8 dt/ha). The biggest seed yield achieved lines of M-10454 mutant (21,3 dt/ha - 28,1 dt/ha). Seed yield of the second mutant M-10464 was lower and amounted to 17,3 dt/ha - 19,9 dt/ha. The lines of M-681 mutant were characterized by the biggest variability of yielding. Their yielding amounted to 4,0 dt/ha - 20,0 dt/ha.

CONCLUSION

Fatty acid composition: oleic, linoleic, linolenic in seeds of the investigated mutants was constant. Despite the changes made by mutagenesis and inbreeding depression, they had good enough agronomy value to use them in breeding.

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