Selection of mutants with high oleic acid content after chemical mutagen treatment of the double low winter oilseed rape

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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. 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 75–80% oleic acid not only improves edible oil but also opens new possibilities for oleochemicals uses.

Mutagenesis played a major role in the development of novel variation for the fatty acid profile of oilseed crops. The first mutation experiment resulting in a substantial modification of the fatty acid composition of crops was started in Germany in 1968 by G. Rakow. The author isolated rapeseed mutants having either reduced or increased linolenic acid concentration (Rakow 1973). More recently, Auld *et al*.(1992) in *B. napus* and *B. rapa*, as well as Wrong and Swanson (1991), Rücker and Röbbelen (1995), Byczyńska *et al*.(1996) and Spasibionek *et al*.(1999) in *B. napus*, developed several mutants with alternations in their concentration of oleic, linoleic and linolenic acid.

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 mutant in successive generations of double low winter oilseed rape are presented.

Materials and methods

The seeds of double low winter oilseed rape strain PN 3756/93 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 were treated with solution of 1% ethyl methanesulphonate (EMS). The seeds of M_2 generation with changed fatty acid composition were treated again with a solution of 5% EMS. In successive generations after mutagen treatment two mutants with changed fatty acid composition were selected.

First screening for polyunsaturated fatty acids was done with the, use of thiobarbituric acid method (TBA) to estimate the level of linolenic acid (McGregor 1974, Byczyńska *et al.* 1994). This method can be applied for screening of large number of seeds in M_2 generation after treatment with the mutagen.

Fatty acid composition of seed oil was estimated by gas chromatography (Byczyńska, Krzymański 1969).

Results

The paper presents selection of mutants from M_2 to $(M_2)_6$ generations with high oleic acid content and decreased linoleic and linolenic acid content after mutagenesis. In $(M_2)_5$ and $(M_2)_6$ generations after mutagen treatment, the mutants with changed and stabilized fatty acid composition were selected (Figure 1-3).

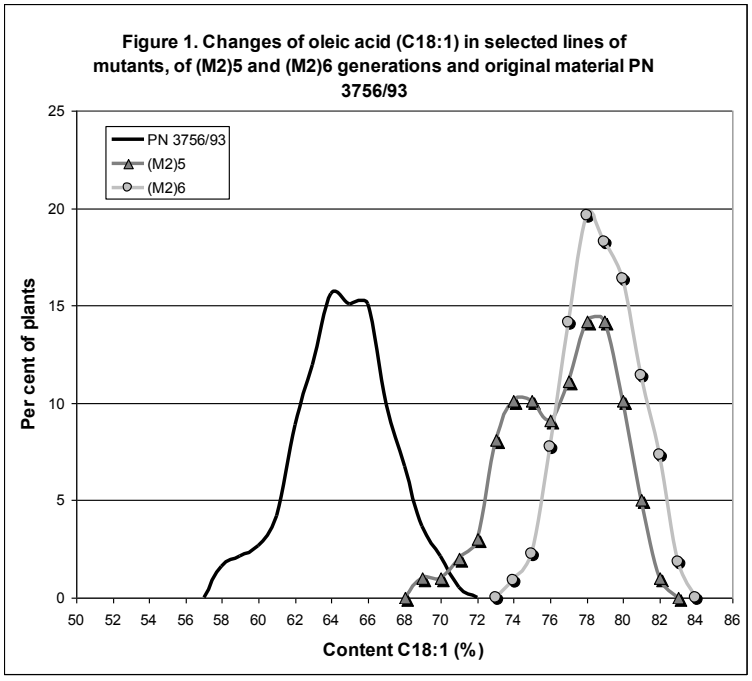


Figure 3. Changes of linolenic acid (C18:3) in selected lines of mutants, of (M2)5 and (M2)6 generations and original material PN 3756/93

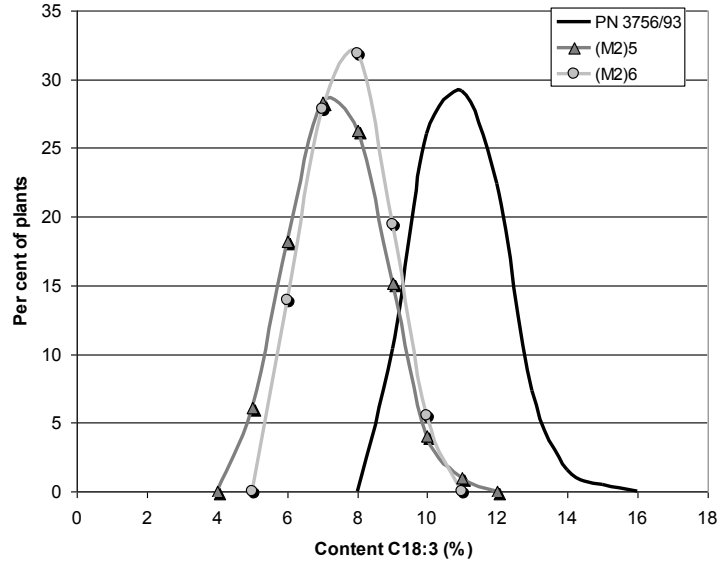
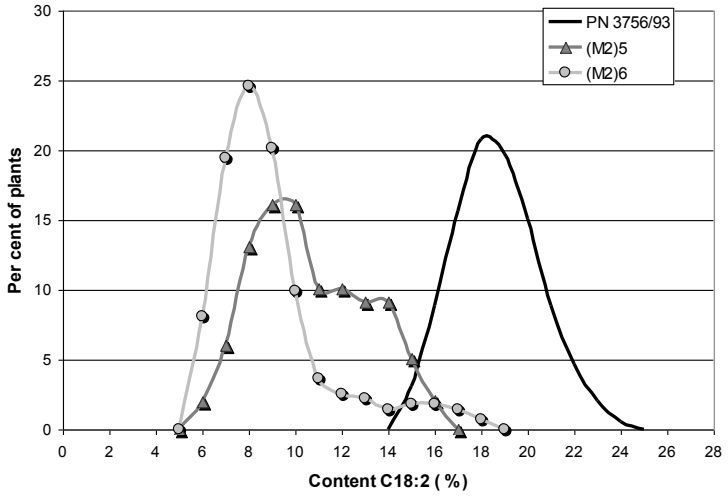


Figure 2. Changes of linoleic acid (C18:2) in selected lines of mutants, of (M2)5 and (M2)6 generations and original material PN 3756/93



The mutants of $(M_2)_6$ generations were characterized by stable high content of oleic acid (mean 78,4% within the range from (74,8% to 81,8%) and decreased content of linoleic acid (7,5%; 5,1- 10,7%) and linolenic acid (7,1%; 8,4-5,9%) (Table1).

Table 1. Content of oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids in seeds of selected lines after treatment with EMS and original material PN 3756/93

Generation	Acid	Original material PN 3756/93					Mutant lines				
		No. of plants	min	max	mean	CoV	No. of plants	min	max	mean	CoV
PN 3756/93	C18:1	64	58,2	69,4	64,1	3,7					
	C18:2		14,6	22,5	18,2	9,4					
	C18:3		8,4	13,3	10,4	8,8					
M2/94	C18:1	28	63,5	69,7	67,2	2,9	330	61,7	79,6	70,3	3,8
	C18:2		14,7	20,2	17,1	8,1		6,8	25	15,1	15,5
	C18:3		7	9,4	8,1	8,1		3	9,6	6,7	13,3
(M2)2/96	C18:1	3	62,7	65,8	64,1	2,4	59	55,7	67,6	62,9	3,9
	C18:2		17,4	19,2	18,1	5,1		16,1	25,1	19,4	9,1
	C18:3		9	11,2	10,4	11,5		7,6	12,3	9,6	10,2
(M2)3/97	C18:1	3	66,6	68,8	67,4	1,8	65	58,6	77,5	66,4	4,7
	C18:2		16,1	18,2	17,2	6,1		7,3	25,1	17,4	16,3
	C18:3		7,3	8,1	7,7	5,2		5,7	10	7,7	12,4
(M2)4/98	C18:1	26	61,8	68,3	65,4	2,4	108	53,1	80,1	66,4	5,9
	C18:2		15,7	20,1	17,7	5,8		8,3	26	18,4	17,8
	C18:3		6,4	10,3	8,9	10,2		5	11,9	8,3	18,9
(M2)5/99	C18:1	20	58,7	67,5	64,4	3,6	**33**	**69,8**	**80,6**	**76,3**	**3,6**
	C18:2		17,3	23,6	19,7	7,6		**5,9**	**15**	**10,2**	**23,3**
	C18:3		7,1	10,8	8,7	12		**5**	**9,3**	**7**	**14,5**
(M2)6/00	C18:1	12	63,8	69,3	66,2	2,4	**73**	**74,8**	**81,8**	**78,4**	**2,2**
	C18:2		15	19,6	17,7	7,5		**5,1**	**10,7**	**7,5**	**13,5**
	C18:3		7,2	9,5	8,6	8,6		**5,9**	**8,4**	**7,1**	**7,9**

Variance analyses confirm stable fatty acids composition in mutated lines (Table 2).

Table 2. Variance analyses for oleic acids (C18:1) conducted after selection of mutated lines in successive generations from M2 to (M2)6 and original material PN 3756/93

Source of variability	Degrees of freedom	Sum of squares	Mean of squares	F
Generation M2				
Mutants versus PN 3756/93	1	242,3	242,3	34,9***
Residual	356	2470,6	6,9	
Total	357	2712,9		
Generation (M2)2a)				
Mutants versus PN 3756/93	1	4,2	4,2	0,71a)
Residual	60	358,6	6	
Total	61	362,8		
Generation (M2)3a)				
Mutants versus PN 3756/93	1	2,8	2,8	0,29a)
Residual	66	632,8	9,6	
Total	67	635,5		
Generation (M2)4				
Mutants versus PN 3756/93	1	19	19	1,48
Residual	132	1686	12,8	
Total	133	1705		
Generation (M2)5				
Mutants versus PN 3756/93	1	1767,8	1767,8	**263,8*** **
Residual	51	341,8	6,7	
Total	52	2109,6		
Generation (M2)6				
Mutants versus PN 3756/93	1	1529,8	1529,8	**539,4*** **
Residual	83	235,4	2,8	
Total	84	1765,2		

*** - significant at the level α=0,001

a) - regress in selection caused by strong winter losses

Conclusion

- Chemical mutagenesis is an effective way to search for new genetic sources of fatty acid variability in winter oilseed rape.
- Changes in fatty acid composition observed in mutants suggested that EMS treatment frequently produces mutagenic changes which reduce activity of desaturase of oleic acid system.
- Selected mutant lines containing about 80 per cent of oleic acid and decreased polyunsaturated fatty acids in seed oil can be very useful in breeding new improved cultivars.
- Obtained mutants with high oleic acid showed that during mutation same genes controlling biosynthesis of oleic acid desaturase were damaged.

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