DEVELOPMENT OF HIGH OLEIC ACID RAPESEED

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ABSTRACT

Chemical mutagenesis was used successfully to induce mutations for increased oleic acid levels in the seed oil of winter oilseed rape. Seeds of 'Wotan' were treated with 2% ethylmethane sulfonate. After screening of about 20,000 M_1 plants, half seed analysis of M_2 seeds, and testing the M_2 and M_3 generations five mutants were identified with 75% to 80% oleic acid in comparison to 61% in 'Wotan'. High oleic acid levels correspond to reduced linoleic acid but also linolenic and palmitic acid. In some mutants the content of polyunsaturated fatty acids was reduced to less than 10%.

INTRODUCTION

Rapeseed oil with about 60% oleic acid, 20% linoleic acid, and 10% linolenic acid is a valuable oil for human nutrition. Due to the relatively high content of polyunsaturated fatty acids, oxidative and fry stability are reduced unless the oil is hydrogenated. Oil quality can be improved by developing varieties with reduced polyunsaturated fatty acids and increased oleic acid contents. Rapeseed oil with more than 80% oleic acid represents not only an improved edible oil but also opens new possibilities for oleochemical uses.

Mutation breeding has already been successful in changing seed oil composition in rapeseed. Mutants with reduced linolenic and increased linoleic acid (Röbbelen, Nitsch 1975) as well as increased oleic acid (Auld *et al.* 1992) were found. Therefore, the aim of the presented study was to develop by mutagenic treatment high oleic acid genotypes from a high yielding well adapted winter oilseed rape variety with canola quality.

MATERIALS AND METHODS

In August 1991 seeds of the winter rapeseed cultivar 'Wotan' were soaked in water for 5 h and than treated for 10 h in a solution of 2% ethylmethane sulfonate (EMS). After this mutagenic treatment the seeds were washed with water for 5 h, dryed and sown into the field. M_2 seeds of about 20,000 open pollinated M_1 plants were harvested in July 1992 and screened for oleic acid content by gaschromatography (fatty acid methyl esters) or near infrared reflexion spectroscopy. From 19 M_1 plants with oleic acid levels higher than 'Wotan' single seeds were analysed by half seed technique (Thies 1971). Embryos with >70% oleic acid were planted in the greenhouse. Since the fatty acid composition in M_2 might have been influenced by the extremely clammy growing conditions during summer 1993 no further selection was carried out in this generation. A total of 298 M_3 families was tested with an average of 7 single plants in the field or in the greenhouse in 1994. Fatty acid composition of M_3 and M_4 seeds was analysed by gaschromatography using 200mg bulked samples. Single seeds from the best families were additionally analysed by half seed technique.

RESULTS

Mutagenic treatment with EMS caused a wide range of phenotypic variation in the M_1 generation. Also Variation of oleic acid content was higher in M_1 than in the untreated control (SD 2.58 vs. 1.89) ranging from 44% to 70% in the M_1 vs. 52%-66% in 'Wotan'. 19 M_1 plants with oleic acid higher than 65% were selected as putative mutants. Increased oleic acid contents could be confirmed for five M_1 families in both, the M_2 and M_3 generation and the M_1 plants Nos. 19508, 19517, 19566, 19646 and 19684 were identified as the most promising mutants. Frequency distribution of mean oleic acid content of M_3 families deriving from these five M_1 plants are shown in Table 1. Mean oleic acid content varied up to 77%. Means and standard deviations for the best M_3 families (Table 2) exhibit variation within the M_3 families, too. Single plants had 56.4% to 80.4% oleic acid indicating that the corresponding M_2 plants had been heterozygous for the mutation. The best M_3 selection originating from mutant 19517 exhibited 80.4% oleic acid, 6.7% linoleic acid, and 4.8% linolenic acid. In half seed analyses of 18 M_4 seeds of this plant, oleic acid ranged from 79.7% to 84.1%. Correspondingly, the polyunsaturated fatty acids level varied from 12.6% to 8.7% ('Wotan' about 30%).

In the mutants increased oleic acid levels were closely associated with reduced linoleic acid (r = -0.93). Correlation between oleic acid and linolenic acid was lower (r = -0.31). Variation of saturated fatty acids (3.8% to 11.0%) was mainly caused by palmitic acid levels. Increased oleic acids were correlated with reduced palmitic acids (r = -0.57). The lowest mean of palmitic acid (2.9%) was observed in a M_3 family derived from the M_1 plant 19566. The highest mean of palmitic acid (8.3%) occurred in a progeny with reduced oleic acid content (53.4%).

Table 1: Frequency distribution of mean oleic acid contents (in % of total fatty acids) in M_3 families derived from the M_1 plants 19508, 19517, 19566, 19646 and 19684 after mutagenic treatment of 'Wotan' (61% oleic acid)

Oleic acid %	19508	19517	19566	19646	19684
59	1		1		
60					
61		1		2	
62		1	1	2	1
63	2			2	1
64	3		2	3	4
65	1		5	3	7
66	4	1	2	5	4
67	1	2	7	3	5
68	2	4	7	2	3
69	1	2	6	3	
70	4	2	7	3	4
71	1	3	5	2	1
72	2	4	6	1	4
73		3	5	2	3
74	1	1	3	1	1
75			2	2	3
76					
77			1		
n[1)]	23	24	60	38	41
mean[2)]	67,3	69,4	69,3	66,7	68,2

1) Number of M_3 families (each family tested with an avarage of 7 plants)

2) Total M_3 mean for M_1 families

Table 2: Oleic acid content (in % of total fatty acids) in the M_2 and M_3 generation of five mutants selected from 'Wotan'

M_1	M_2			M_3 mean		best M_3 plant		
plant no.	plant no.	C18:1	n[1)]	C18:1	SD	C18:1	C18:2	C18:3
19508	14707	76,7	54	72,5	3,76	**78,2**	7,0	7,3
19517	14564	78,5	57	71,0	3,22	**80,4**	6,7	4,8
19566	14692	74,0	10	74,3	2,34	**77,4**	7,8	6,6
19646	14851	71,2	9	72,6	3,56	**77,7**	8,3	6,1
19684	14931	71,5	10	75,4	1,52	**77,5**	7,5	7,3
Wotan			11	61,5	1,84			

1) Number of M_3 plants tested

DISCUSSION

After mutagenic treatment of 'Wotan' it was possible to select new genotypes with stable oleic acid levels being 15% to 20% higher than 'Wotan'. The identified mutants originated from five M_1 plants and therefore at least five independent mutagenic events must have happened. Inheritance studies are in progress to find out how many genes are responsible for the changed fatty acid composition in each mutant and whether there are genetic differences between these mutants. If different genes are changed by mutation, crosses between the mutants should result in recombinants with more than 80% oleic acid content.

Increase of oleic acid content in the seed oil results from a reduced level of polyunsaturated fatty acids. Thus, genes responsible for oleate desaturation are to be changed. As shown for other high oleic acid mutants in rapeseed (Kinney *et al.* 1994) this may be accomplished by mutation of a structural gene encoding for cytosolic oleate desaturase. Because of the complex biosynthetic pathway of triacylglycerol, other enzymes may be involved, too, e.g. transacylases.

ACKNOWLEDGEMENT

This work was supported by the EC Commission under the ECLAIR program by contract No. SONCA AGRE-CT 90-0039.

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