Quantity and quality effects in fatty acid segregation at winter oilseed rape hybrids (*Brassica napus* L.)

Jan Krzymanski, Marcin Matuszczak, Teresa Cegielska-Taras, Laura Szala, Irena Tokarczuk

Department of Oil Crops, Institute of Plant Breeding and Acclimatisation, Strzeszyńska 36, Poznań, 60-479, Poland
Email:krzym@nico.ihar.poznan.pl

Abstract

Segregations of fatty acid composition in F_2 generations of rapeseed hybrids investigated till now had rather continuous character as typical for metric traits. Clearly defined zero erucic fraction occurring in relation 1:15 was found only at segregation in cross combinations "high erucic×zero erucic". (Harvey, Downey 1964, Kondra, Stefansson 1965, Krzymański at al. 1967, Krzymański, Downey 1968, Krzymański 1970, Jönsson 1977, Anand, Downey 1981, Chen, Heneen 1989, Chen, Beversdorf 1990, Scheffler at al. 1997, Barret at al. 1998, Fourmann at al. 1998, Lühs at al. 1998).

This continuous distribution observed for segregation of fatty acids may be a result:

- of control of these traits by polygenic genes working on cumulative manner or
- of masking the effects of main genes with small changes made by a number of modifying genes or
- of interference of main gene effects with effects of non heritable variability like: physiological variation, environment influences, chemical analyses errors, non proper sampling.

To explain the problem more exact study was conducted on segregation of fatty acids in hybrids of winter oilseed rape cultivated in Poland.

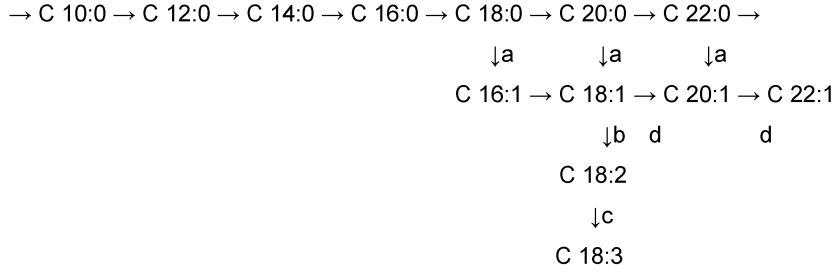
Key words: rapeseed, seed oil, fatty acids, biosynthesis, inheritance, segregation

Methods

The study was conducted on population of 291 doubled haploid lines (DH). These lines were obtained with the use of "in vitro" culture of microspores collected from F_1 hybrid plants (Cegielska-Taras at al. 1997**,** 2002). The hybrid was obtained in cross combination: "high erucic, low glucosinolate line"×"zero erucic, high glucosinolate line". Parental lines differ greatly in origin and chemical composition. The population of DH lines was produced for constructing the genetic map of winter oilseed rape cultivated in Poland. It was expected that use of doubled haploid lines allow to obtain much better resolution than with inbreed lines.. DH lines are not only homozygous but also the segregation ratio should be more simple. Seeds from two self pollinated plant per line were collected and analysed. Chemical analyses of fatty acid composition in seed oil were made with gas liquid chromatography of their methyl ethers. Statistical analysis of segregation process were conducted using Microsoft Excel 97 and Statistica 5.1. programs.

Results and Discussion

Biosynthesis of fatty acids in ripening seeds of oilseed rape has following pathways (Yadav et al. 1993, Cassagne at al. 1994, Slabas at al.1995, Lassner at. al. 1995,1996, Clemens at al. 1997, Millar, Kunst 1997) shown bellow:



where:

C 18:1 – denote acid with 18 carbon atoms in chain and with 1 double bond (oleic)

a = Δ9-desaturase of stearic acid

b = Δ12-desaturase of oleic acid

c = Δ15-desaturases of linolic acid

d = elongases of oleic and eicosenoic acids

Elongation of oleic acid chain is blocked in seeds of zero erucic rapeseed. Genetic source of this trait was found as natural mutant in Liho variety of spring rapeseed. (Stefansson at al. 1961,1964,).

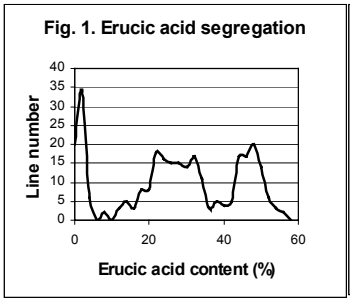
Variability of fatty acids in studied population of 291 DH lines is shown in table 1.

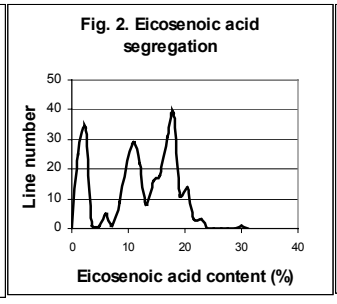
Table 1. Fatty acid segregation in population of 291 DH lines of rapeseed (per cent of total fatty acid)

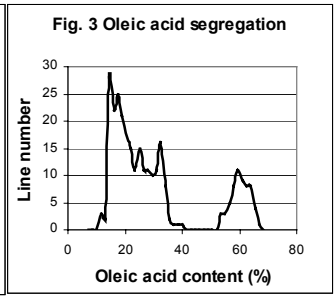
	Palmitic	Stearic	Oleic	Linolic	Linolenic	Eicosenoic	Erucic
Mean	4,506	1,099	29,467	18,045	9,196	11,429	26,249
Minimum	3,0	0,3	10,5	11,7	4,4	0,4	0,0
Maximum	7,0	1,9	65,9	29,2	13,9	23,2	54,8
C of V.	16,493	25,774	55,923	18,841	16,015	55,016	63,862
s	0,226	0,133	1,386	0,833	0,552	1,226	0,854
s_d	0,320	0,188	1,960	1,178	0,780	1,734	1,208
LSD	0,640	0,375	3,920	2,356	1,560	3,468	2,415

where: s = error of mean for line (two replication)
s_d = error for difference between two line
LSD = least significant difference between means of two lines (P=0,05)

With the use of DH lines it was possible to isolate not only zero erucic fraction, but also to split the rest of population in two fractions: high erucic and intermediate. Intermediate fraction was irregular but it was difficult to state two subfractions connected with genes from *Brassica* rapa and *Brassica* oleracea. Frequency distribution of erucic, eicosenoic and oleic acids are shown on Fig. 1, 2 and 3.







These histograms were made with resolution approximately equal to standard deviation for line average. Histogram fractions contain many lines so differences in average values between them were very significant as it is shown in table 2.

Table 2. Least significant differences ($LSD_{0,05}$) for fatty acid contents by comparing fraction containing different number of lines (per cent of fatty acid)

Number of line in fraction	Fatty acid		
	Oleic	Eicosenoic	Erucic
1	3,920	3,468	2,415
2	2,772	2,452	1,708
3	1,600	1,416	0,986
4	0,800	0,708	0,493
5	0,358	0,317	0,220

The ratios among the fractions set according frequency distribution of erucic, eicosenoic and oleic acid contents showed some deviations from theoretical segregation ratio 1:2:1. It was found:

- zeroerucic fraction 60 lines
- intermediate fraction 145 lines
- high erucic fraction 86 lines.

Calculated χ^2 has value.4,649 with df=2 and probability of larger χ^2 is only P=0,098 what means that deviation from theoretical ratio is significant. This phenomenon can occur in DH lines populations. High erucic microspores (wild type) were probably preferred in haploidisation process.

Above analysis did not allow to split intermediate fraction into two subfractions connected probably with genes originated from *Brassica* oleracea (E_o) and from *Brassica* rapa (E_r). Four classes of segregates (00 00), (00 E_oE_o), (E_rE_r 00) and (E_rE_r E_oE_o) with ratio 1:1:1:1 can be expected in our population of DH lines. Method of multivariate grouping with 4 means were used to split DH lines in four groups according to contents of erucic, eicosenoic and oleic acids. Mean values of erucic, eicosenoic and oleic acids for these groups are shown in table 3 and their analyses of variance in table 4.

Table 3. Mean contents of fatty acids in calculated groups (%)

Acid	Group 1	Group 2	Group 3	Group 4
n=	60	69	73	89
Erucic	0,56	19,54	29,69	45,95
Eicosenoic	1,33	16,67	16,56	9,97
Oleic	59,51	29,74	21,42	15,61

Differences among groups according tree examined fatty acids are highly significant. Calculated χ^2 for these groups has

value.6,058.with df=3 and probability of larger χ^2 equal to P=0,109 what means that deviation from theoretical ratio are not significant on the level α =0,05. Obtained groups can be used for genes mapping (gen loci) not only for calculation of quantitave (metric) trait loci (QTL).

Table 4. Analyses of variance for fatty acids in groups

Acid:	SS	df	SS	df	F	p
Erucic	78110,35	3	3377,734	287	2212,299	0
Eicosenoic	10122,2	3	1342,988	287	721,0468	0
Oleic	75993,61	3	2757,898	287	2636,085	0

Conclusions

1) Due to higher resolution it was possible to separate not only zero erucic fraction but also to split the rest of DH line population in two clearly separated fractions: high erucic and intermediate.

2) Intermediate fraction did not have visible separation in two subfractions. This suggest that genes originated from *Brassica* oleracea and *B. rapa* have similar control in synthesis of erucic and eicosenoic acids..

3) The number of DH lines in fractions estimated according to contents of erucic and eicosenoic acids was as follow: zero erucic – 60 lines, intermediate – 145 lines, high erucic – 86 lines. It shows some deviation from theoretical ratios 1:2:1. High erucic microspores were probably preferred in tissue culture.

4) It was not possible to split intermediate fraction in two subfraction using one factor analysis. This separation was done with the use the method of multivariate grouping with four means. Four groups were obtained according to contents of erucic, eicosenoic and oleic acids. These groups contain 60, 69, 73 and 89 lines. Segregation hypothesis 1:1:1:1 cannot be rejected by χ^2 test but some deviation on benefit to wild type is also visible.

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