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Fatty acid inheritance in microspore-derived Populations of spring rapeseed (*Brassica napus* L.)

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Summary. The inheritance of major fatty acids in seed triglycerides was studied in three homozygous microspore-derived populations of spring rapeseed (*Brassica napus* L.). Crosses were made among parents with contrasting amounts of erucic, oleic, linoleic and linolenic acids. Microspores from F_1 plants were cultured, and haploid plants were colchicine-doubled to provide homozygous populations reflecting F_1 gametic arrays for fatty acid genotypes. Segregation ratios of the gametic arrays for specific fatty acid contents were compared to hypothetical models by the Chi-square test. Segregation pattern confirmed that erucic acid levels were controlled by two major loci, each having two alleles with additive effects. Oleic acid segregation indicated control of accumulation by at least two segregating genetic systems, one acting on chain elongation and the other involving desaturation. Accumulations of erucic acid and oleic acid were influenced by the same two loci, which control the chain elongation steps leading from oleic acid to erucic acid. Oleic acid was further influenced by at least two additional segregating loci involved in control of desaturation of oleic acid to form linoleic acid. Segregating alleles at loci involved in desaturation had a much smaller influence on oleic acid content than alleles segregating at loci controlling, the elongation of oleic acid to erucic acid. In a population free of erucic acid, the segregation pattern of linoleic acid levels fit a model involving segregating alleles at two loci. In contrast, segregation for linolenic acid content fits a three-locus additive model. In this study, microspore culture technology provided a rapid method of defining F_1 gametic segregation for inheritance analyses.

Key words: Microspore-derived population – Fatty acids – Inheritance – *Brassica napus*

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Introduction

Brassica napus L. and *B. campestris* L. are the two major species of oilseed rape. *Brassica* seeds commonly contain 40%–44% oil on a dry-weight basis and produce an oil-free meal with 38%–41% protein (Downey and Rakow 1987). The quality of rapeseed oil is determined by its constituent fatty acids. Rapeseed oil contains considerable amounts of erucic, oleic, linoleic and linolenic acids. Erucic acid is a nutritionally undesirable monoenoic fatty acid, while linolenic acid, a trienoic fatty acid, is unstable and easily oxidized to cause off-flavour of oil. In contrast, linoleic acid, a precursor of various prostaglandins, is an essential fatty acid (Robbelen and Nitsch 1975). The breeding objectives for improved seed oil quality include the elimination or a reduction in content of erucic acid and linolenic acid in the seed oil with commensurate increases in linoleic acid and oleic acid content.

Employing conventional breeding approaches, Harvey and Downey (1964) determined that erucic acid in oil from *B. napus* was controlled by two genes acting in an additive manner. The inheritance of the other unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid are less understood.

Since the reports of Lichter (1981, 1982) on *Brassica* anther and microspore culture, refinements to anther and microspore culture procedures have led to the expanded utilization of haploids to produce homozygous breeding lines. The F_1 gametic array of haplotypes in F_1 (plants) microspore-derived populations provide an opportunity for rapid and efficient genetic investigations by eliminating heterozygous loci characteristic of early segregating generations from inbreeding or backcrossing a heterozygous hybrid. The current study was conducted to evaluate the genetic control of major fatty acid contents

in seed oil of *B. napus* on the basis of F_1 gametic segregation reflected in F_1 microspore-derived homozygous populations. Further, a discussion of the influence of specific segregating loci based on correlation analyses is provided.

Materials and methods

Parent materials and crosses

Three spring rapeseed (*B. napus*) lines, Reston-S_1, R83-12-S_1 and GS-272-S_1, derived from germ plasm provided by Dr. B. R. Stefansson, University of Manitoba, were crossed reciprocally with G-231-S_1 in a controlled growth-room. The line G-231-S_1 is a homozygous Guelph experimental line with a typical canola fatty acid profile. Crosses were used discriminantly for genetic analysis of specific fatty acids because of the contrasting amounts of each specific fatty acid in their parental lines (Table 1). Erucic acid (22:1) content was evaluated in the progeny of the cross (G-231-S_1 × Reston-S_1). Oleic acid (18:1) segregation was evaluated in the progeny of two crosses: (G-231-S_1 × Reston S_1), where oleic acid co-segregated with erucic acid, and (G-231-S_1 × R83-12-S_1), where both parents were low in erucic acid. Linoleic acid (18:2) content was studied in the progeny of the cross (G-231-S1 × R83-12-S_1). Linolenic acid (18:3) segregation was evaluated in the progeny of the cross (G-231-S_1 × GS-272-S_1).

Production of microspore-derived populations and selfed progenies

Microspore-derived populations for each fatty acid of interest were produced through in vitro culture of microspores from F plants of each cross, using the method described by Polsoni et al. (1988). Doubled haploid plants were self-pollinated to provide reciprocal populations of microspore-derived pure lines for each specific cross.

Field seed increase and fatty acid analysis

For each fatty acid investigated, pure line progeny of the microspore-derived populations were grown at the Elora Research Station in replicated progeny rows in a completely randomized design (with three replications) along with their respective parents. The populations are composed of 84 lines from G-231-S × Reston-S_1 for erucic acid analysis, 78 lines from G-231-S_1 × GS-272-S_1 for linolenic acid analysis and 82 lines from G-231-S_1 × R83-12-S_1 for linoleic and oleic acids analysis. Chemical analysis of fatty acid content, using the GLC method described by Siebel and Pauls (1989), was conducted on a composite of 20 seeds from each of 5 self-pollinated plants per replicate of progeny rows. Fatty acid content was expressed as a percentage of total fatty acid content.

Statistical analysis

Microspore-derived lines were utilized to establish phenotypic classes for specific fatty acid contents on the basis of population distribution. Since all the lines produced from microspores are homozygous, lines with distributions around the parent values were classified as a high or low content group; lines with distributions between parent values were classified as an intermediate content group. The Chi-square test was used to evaluate the genetic models proposed for fatty acid interitance in microspore-derived populations. The proposed genetic models were based on F_1 gametic arrays (Table 2).

Table 1. Parental seed fatty acid composition

Parental lines	Main fatty acid content (%)			
	Oleic	Linoleic	Linoleic	Erucic
G-231-S_1	60.0	22.8	9.5	0.0
R83-12-S_1	69.2	11.1	8.9	0.0
GS-272-S_1	69.1	18.9	2.6	0.0
Reston-S_1	20.2	14.4	11.3	42.3

Table 2. Hypothetical genetic model for F_1 gametic arrays

n[a]	Genotype			F_1 gametic array[b]	Phenotypic ratio[c]
	P_1	P_2	Hybrid		
2	*AABB*	*aabb*	*AaBb*	*AABB*	1
				AAbb	2
				aaBB	
				aabb	1
3	*AABBCC*	*aabbcc*	*AaBbCc*	*AABBCC*	1
				AABBcc	3
				AAbbCC	
				aaBBCC	
				AAbbcc	3
				aaBBcc	
				aabbCC	
				aabbcc	1

[a] n is the number of gene pairs
[b] As doubled haploids (e.g. gamete AB = doubled haploid genotype *AABB*)
[c] Phenotypic ratio from population of pure lines (due to additive effects)

Table 3. Relative percentage fatty acid (FA) range definitions for groupings based on distribution pattern of microspore-derived populations

FAs	Low (%)	Intermediate (%)	High (%)	Population source
Oleic[a]	18–30	31–50	51–69	G-231-S_1 × Reston-S_1
Oleic[b]	58–62	63–67	68–74	G-231-S_1 × $R_{83\text{-}12}$-S_1
Linoleic	10–13	14–19	20–25	G-231-S_1 × $R_{83\text{-}12}$-S_1
Linoleic	2–3.4	3.5–5.4; 5.5–8.4	8.5–10	G-231-S_1 × GS-272-S1
Erucic	0–2	20–35	36–45	G-231-S_1 × Reston-S_1

[a] Oleic acid content in a population with erucic acid co-segregation
[b] Oleic acid content in a population without erucic acid co-segregation

Table 4. Chi-square test for gametic segregation ratios for fatty acid content in *B. napus*

FAs	Number of plants					Ratio	χ^2	*P*
		Low	Intermediate	High	Total			
Oleic[a]	O	21	38	23	82	1:2:1	0.54	0.8–0.9
	E	20.5	41	20.5	82			
Oleic[b]	O	20	39	25	84	1:2:1	1.02	0.5–0.8
	E	21	42	21	84			
Linoleic[c]	O	20	39	23	82	1:2:1	0.41	0.8–0.9
	E	20.5	41	20.5	82			
Linolenic[d]	O	12	27; 30	9	78	1:3:3:1	0.77	0.8–0.9
	E	9.75	29.25; 29.25	9.75	78			
Erucic[e]	O	23	40	21	84	1:2:1	0.29	0.8–0.9
	E	21	42	21	84			

O, Observed value; E, expected value
[a] From cross G-231-S_1 × R83-12-S_1 (population withouth erucic acid co-segregation)
[b] From cross G-231-S_1 × Reston-S_1 (population with erucic acid co-segregation)
[c] From cross G-231-S_1 × R83-12-S_1
[d] From cross G-231-S_1 × GS-272-S_1
[e] From cross G-231-S_1 × Reston-S_1

Results

1) Genetic model of fatty acid inheritance

Range definitions for grouping of lines with various contents of specific fatty acids in corresponding populations are shown in Table 3 in accordance with the criteria described above. The number of individual lines falling into the phenotypic classes for each fatty acid are given in Table 4. The Chi-square test was used to fit the genetic model on the basis of Table 2.

The inheritance of erucic acid was evaluated in the progeny of a cross between low × high erucic acid parents. The distribution of erucic acid followed a phenotypic ratio of 1 low : 2 intermediate : 1 high erucic acid lines fitted by Chi-square test (Table 4), which indicated that in this population erucic acid was controlled by alleles at two loci.

The inheritance of oleic acid was investigated in two different populations, i.e. with and without erucic acid co-segregation. In the population co-segregating for oleic acid and erucic acid, segregation for oleic acid involved three broad phenotypic classes (Table 3) in a ratio of 1 low : 2 intermediate : 1 high oleic acid content (Table 4). This indicates that oleic acid is controlled by two loci with two alleles per locus. In the population without erucic acid co-segregation, a separate segregation pattern with much narrower phenotypic classes was apparent (Table 3), which also fitted a 1 low : 2 intermediate : 1 high ratio (Table 4), indicating that at least two additional loci control oleic acid content.

The segregation of linoleic acid content fell into three phenotypic classes (Table 3) that were consistent with a 1 low : 2 intermediate : 1 high ratio (Table 4). Therefore, a genetic model involving alleles at two loci is proposed for linoleic acid inheritance.

The segregation for linolenic acid appeared to involve at least four phenotypic classes (Table 3). Chi square tests indicated that the segregation patterns were consistent with a 1 low : 3 low intermediate : 3 high intermediate : 1 high ratio (Table 4). Segregation in this population supports the hypothesis that linolenic acid level is controlled by alleles at three loci.

2) Correlations involving fatty acid content

Correlations among specific fatty acid contents were dependent on the presence and absence of segregation for eicosenoic and erucic acids (Table 5). In the population segregating for erucic acid, both erucic and eicosenoic acids were negatively correlated with oleic acid and linoleic acid; eicosenoic acid was also negatively correlated with linolenic acid. Oleic acid content was positively correlated with linoleic acid content, and linoleic acid content was positively correlated with linolenic acid content. Eicosenoic acid and erucic acid were significantly correlated in the population (G-231-S_1 × Reston-S_1). Within this population, however, the levels of these fatty acids were positively correlated in the sub-population having an erucic acid content of less than 25%, but were negatively correlated in the sub-population having an erucic acid content over 25% (Table 5).

Table 5. Correlation among fatty acids in a population segregating for erucic acid (G-231-S_1 × Reston-S_1) and in a population free of erucic acid (G-231-S_1 × GS-272-S_1) in *B. napus*[a]

	Linoleic	Linolenic	Eicosenoic	Erucic
Oleic	0.236* (−0.981**)	−0.150 (−0.502**)	−0.503**	−0.93**
Linoleic		0.483** (0.318**)	−0.417**	−0.412**
Linolenic			−0.242*	0.033
Eicosenoic				0.91**; −0.72**[b]

* $P \leq 0.05$ by t-test; ** $P \leq 0.01$ by t-test

[a] Numbers in brackets are the correlation coefficients in a population free of erucic acid %

[b] ($r = 0.91$** in a population with 0–25% erucic acid; $r = -0.72$** in a population with >25% erucic acid)

In populations free of both erucic acid and eicosenoic acids, oleic acid content was negatively correlated with linoleic and linolenic acid contents, while linoleic and linolenic acids were positively correlated.

Discussion

Microspore culture offers a rapid means of reaching homozygosity for segregating characters through chromosome doubling. Efficient genetic investigations can be carried out on specific characters, based on the assumption that microspore-derived populations reflect an unbiased (random) sampling of segregating F_1 gametes an assumption that has been confirmed in a related study of fatty acid profiles (Chen 1988). Observed and hypothetical segregation ratios for genes affecting major fatty acids were compared for goodness of fit by chi-square analysis.

In this study the inheritance pattern of erucic acid in microspore-derived populations conforms to the inheritance pattern of Harvey and Downey (1964), who used a conventional breeding method, and also to that of Siebel and Pauls (1989), who evaluated populations of *B. napus* microspore-derived spontaneous diploids. Among the populations evaluated, segregation for oleic acid was controlled by at least two gene systems, each containing two loci. One of these two gene systems has a major effect on both oleic acid and erucic acid contents, as the segregation of these two acids is interdependent; high oleic acid content was always associated with low erucic acid and vice versa ($r = -0.93$**). The other gene system which affects oleic acid is of minor importance in comparison to the gene system involving the co-segregation of erucic acid. We propose that this minor gene system explains the reversal in correlation between oleic acid and linoleic acid when segregation for erucic acid is present ($r = 0.24$*) or absent ($r = -0.98$**). The strong negative correlation between erucic acid and oleic acid content suggests that the alleles at the two loci in the major gene system act at the elongation step of the 18:1 → (20:1) → 22:1 pathway. The alleles at the two loci in the minor gene system appear to control the desaturation step of 18:1 → 18:2, as reflected by the strong negative correlation between oleic acid and linoleic acid in the absence of erucic acid segregation.

The complexity of the inheritance of linolenic acid may be the result of two different synthetic pathways. Linolenic acid (18:3) can be synthesized either from the desaturation of 18:2 or possibly by the elongation of 16:3 (Thompson 1983). Segregating alleles at three loci appear to influence 18:3 levels in G-231-S_1 × GS-272-S_1. These loci may control either or both of these pathways of linolenic acid synthesis. However, the positive correlations between 18:2 and 18:3 in these populations suggest that a portion of the segregation affecting 18:3 is not controlling desaturation of 18:2 to 18:3.

A general investigation of correlations among fatty acids in the populations indicated that the correlations were altered to some extent by the presence or absence of erucic acid. In the population with erucic acid, eicosenoic acid showed a high negative correlation with all of the C18 fatty acids. Earlier studies indicated that while eicosenoic acid has the same genetic controls as erucic acid, the alleles involved have an additive effect on erucic acid, but a dominant effect on eicosenoic acid (Kondra and Stefansson 1965). Therefore, the correlation between the two acids was expected to be highly significant. In the present study the correlation between eicosenoic acid and erucic acid content is positive at levels of erucic acid up to 25%, but becomes negative at higher levels of erucic acid up to 25%, but becomes negative at higher levels of erucic acid (Table 4), which agrees with the results of Jonsson (1977). The impact of erucic acid segregation on the correlation among other fatty acids was evident. The positive correlation between oleic acid and linoleic acid in the population segregating for erucic acid changed to a negative correlation in the population free of erucic acid. This reversal indicates that genetic control of oleic acid desaturation may be apparent only in the absence of erucic acid segregation. This minor gene system is not apparent in the presence of erucic acid segregation, perhaps as a reflection of its impact on oleic acid compared to the alleles at loci controlling chain elongation from oleic acid. The consistent positive correlation between linoleic acid and linolenic acid in the low and the high erucic acid populations suggests that alleles involved in controlling desaturation of linoleic acid to linolenic acid are not affected by the alleles influencing erucic acid/oleic acid levels.

Microspore culture technology provides a rapid method of defining F_1 gametic segregation for inheritance analyses. In the current study the F_1 gametic segregation assays reflected in microspore-derived populations (homozygous lines) fit previous models for erucic acid inheritance. Segregation of microspore-derived populations also provided new information on the inheritance of oleic, linoleic and linolenic acids in *B. napus*.

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