THE IDENTIFICATION OF GENE LOCI AND ALLELIC SERIES CONDITIONING THE INHERITANCE OF ERUCIC ACID IN RAPESEED (*BRASSICA NAPUS* AND *BRASSICA CAMPESTRIS*)

A Thesis

Submitted to the Faculty

of

Graduate Studies

by

Nancy Jane Romanow

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

May 1978

THE IDENTIFICATION OF GENE LOCI AND ALLELIC SERIES CONDITIONING THE INHERITANCE OF ERUCIC ACID IN RAPESEED (*BRASSICA NAPUS* AND *BRASSICA CAMPESTRIS*)

BY

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

ACKNOWLEDGMENTS

I would like to express my appreciation to my advisor, Dr. B.R. Stefansson, for his guidance in the preparation of this manuscript.

The staff in the chemistry lab were also very helpful with chemical analyses and I am extremely grateful to Mary-Jane Blank for her assistance in the latter stages of fatty acid analysis.

I would also like to thank Dr. A.K. Storgaard for her help and encouragement throughout the past few years.

Last, and most important, I thank my husband, Don, for his continual support during this time.

ABSTRACT

Romanow, Nancy Jane, M.Sc., The University of Manitoba, May, 1978. The Identification of Gene Loci and Allelic Series Conditioning the Inheritance of Erucic Acid in Rapeseed (Brassica napus and Brassica campestris).

Major Professor: Dr. B.R. Stefansson

The inheritance of erucic acid in the seed oil from rape (Brassica napus L.) and turnip rape (Brassica campestris L.) appears to be controlled by two gene pairs and one gene pair, respectively. These genes lack dominance and act in an additive manner. This project was undertaken to determine the number of loci involved in erucic acid inheritance, to find the source of the loci and to identify the alleles on each locus in rape and turnip rape.

Monogenic lines with a single gene pair conditioning erucic acid were developed from four strains of rape and three strains of turnip rape in a common rape background. Two monogenic lines with genes on different loci were used as testers. The genes for erucic acid in one of these lines were derived from turnip rape and labelled E_cE_c. The other tester, derived from rape was identified as containing the locus from B. oleracea and labelled E_oE_o. The F_2 from crosses involving the two testers and the other monogenic lines were used to identify the loci in the other lines.

The E_cE_c locus was identified in all monogenic lines derived from turnip rape while both the E_oE_o and E_cE_c loci were identified in

monogenic lines derived from rape. There was no evidence for a third locus.

Allelic series were observed for both loci. The contributions of alleles on the E_cE_c locus were 3-4%, 7-8% and 10-12% erucic. Contributions of the alleles on the E_oE_o locus were 6%, 7.5% and 9-10% erucic acid.

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I. INTRODUCTION

The acceptability of a vegetable oil for either edible or industrial purposes is largely determined by its fatty acid composition. Oils with a large proportion of long chain fatty acids (20 carbons or more) are usually used for industrial purposes whereas those containing shorter chain fatty acids (18 carbon atoms or less) are normally used as edible oils.

Members of the Cruciferae family, particularly rapeseed and mustard, contain substantial amounts of erucic acid, a mono-unsaturated fatty acid with 22 carbon atoms (C22:1). Numerous experiments indicate that animals fed large amounts of their calories from rapeseed oil developed physical abnormalities (Mattson, 1973). These include an accumulation of fat in the heart muscle and similar changes in the skeletal muscle. This occurrence of lipidosis was attributed to an accumulation of triglyceride with erucic acid constituting a large portion of the fatty acids (Mattson, 1973). These effects were observed when the experimental animals ingested a considerable amount of rapeseed oil between 10 and 60% of the diet. These amounts are substantially higher than those likely to be attained in the human diet. Also, human babies are not fed any appreciable amount of the oil. This suggests that the lipidosis observed would not be manifested in humans. However, due to the effects of the erucic acid on animals a decision was made at the International Rapeseed Conference, 1970, which stated

that there should be a gradual changeover to new varieties low in erucic acid content in all rapeseed producing countries.

Stefansson et al. (1961) isolated Brassica napus (rape) plants free from erucic acid. Low erucic acid oil was later isolated from Brassica campestris (turnip rape) lines (Downey, 1964). The genetic control achieved over erucic acid permitted a rapid changeover to low erucic acid varieties in Canada. This low erucic acid content was later combined with a low glucosinolate content in the meal. Other important quality characteristics which are still to be combined with the low erucic, low glucosinolate characteristics include a high content of linoleic acid, a low content of linolenic acid and a yellow seed coat.

Knowledge of the inheritance of erucic acid facilitates breeding programs aimed at improving the oil quality in rapeseed, for both rape and turnip rape. Previous studies have indicated that the erucic acid content in rape is controlled by two additive gene pairs lacking dominance (Downey and Craig, 1963; Harvey and Downey, 1964; Stefansson and Hougen, 1964; Kondra and Stefansson, 1965). The erucic acid content in turnip rape, however, was found to be conditioned by one gene pair (Dorrell and Downey, 1964).

This rather simplified picture of erucic acid inheritance has lately been subject to scrutiny and the suggestion has been made that, at least in turnip rape, a third locus may be involved (Jönsson, 1972).

This project was designed to determine the number of loci involved in the inheritance of erucic acid in the summer forms of both rape and turnip rape, the source of the loci and the contribution of alleles controlling erucic acid at each locus.

II. LITERATURE REVIEW

Before the changeover to varieties with low erucic acid content, the erucic acid levels of oil from summer rape ranged from 35 to 50%. The erucic acid content for the oil of summer turnip rape was between 20 and 50%. Elimination of this major constituent of the oil led to research concerning the inheritance of erucic acid. The literature pertaining to each species will be discussed separately.

A. Rape: Brassica napus

Brassica napus, 2n = 38, is an amphidiploid resulting from natural crossing between Brassica oleracea L., 2n = 18, and Brassica campestris, 2n = 20. There is erucic acid in the seed oil from both of these species, therefore, each species has at least one gene for erucic acid. Thus, rape must have at least two genes conditioning erucic acid content. Several genetic studies have been reported for both summer and winter rape.

Downey and Harvey (1963) reported that the fatty acid composition of the seed of summer rape was controlled by the embryo rather than the maternal parent. This was confirmed by Kondra and Stefansson (1965). Harvey and Downey (1964) postulated that two genes displaying little or no dominance and acting in an equal and additive manner condition the erucic acid content of varieties of summer rape. This theory was

supported with their data from F_2, F_3 and backcross populations, along with the results from the BCF_1 population reported by Kondra and Stefansson (1965). The expected F_2 ratio based on this hypothesis would be 1:4:6:4:1. Since the ratio tended to approach 1:4:11, the following explanation was suggested; each gene contributed 9 to 10% erucic acid, therefore, the range of each phenotype would increase as the number of genes for erucic acid in the genotype increased. This caused the higher three classes to become indistinguishable and thus the ratio tended to approach 1:4:11.

Further research on the genetics of erucic acid in biennial rapeseed produced slightly different results. Krzymanski and Downey (1969) studied the inheritance of erucic acid in biennial rape and in the summer rape variety Bronowski. Results of the F_2 families analyzed led these authors to postulate that one gene pair displaying little or no dominance and acting in an additive manner conditioned the erucic acid content of the oil in these winter rape strains. However, Jönsson (1977) reported that the erucic acid content in Bronowski is controlled by genes located at two loci. Studies with varieties Moana and Rangi indicated that the erucic acid content is controlled by one and two genes, respectively (Lammerink and Morice, 1971). The genes acted in an additive manner. In Rangi each allele contributes 8 to 13% erucic acid when in a single dose; in Moana each gene contributes 7 to 18% erucic acid.

A relatively large number of homozygosity levels for erucic acid have been found in rape. Krzymanski and Downey (1969) reported five alleles found in seed oils from summer rape and summer turnip rape. These included a gene for the absence of erucic acid, an allele

reported by Harvey and Downey (1964) and Kondra and Stefansson (1965) which contributed approximately 10% erucic, an allele for 15% and one for 30% (Dorrell and Downey, 1964), and an allele for 3.5% erucic (Krzymanski and Downey, 1969).

Jönsson (1977) reported on the relatively large number of homozygosity levels for erucic acid, and suggested that there are more levels than reported. Further, the paper states that for erucic acid contents of up to 30% the alleles have an additive effect with respect to erucic acid content while at higher concentrations partial dominance is more common.

The literature generally supports a two gene system with a multiple allelic series for control of erucic acid in summer rape. While two loci and several alleles have been reported, the alleles have not been placed in their respective allelic series.

B. Turnip Rape: Brassica campestris

Brassica campestris, 2n = 20, has erucic acid in its seed oil and therefore must have at least one gene for erucic acid.

Dorrell and Downey (1964) studied the mode of inheritance of erucic acid in summer turnip rape. The erucic acid content of F_1 seeds was intermediate between the two parents which indicated that in this species, as in summer rape, the fatty acid composition was under embryonic and not maternal control. Although there was some overlapping of classes, the ratios for erucic acid from F_2, F_3 and backcross generations indicated that erucic acid inheritance is controlled by a single major gene with alleles lacking dominance and acting in an additive manner.

Jönsson (1972) suggested that the inheritance of erucic acid is somewhat more complex. An unusual distribution found in F_1 seeds from the variety Torpe yielded a group with a mean of 27% erucic and another with a mean of 10% erucic. As these differences in erucic acid content were too large to be explained on the basis of environment alone, the results point to either control by two gene pairs or a series of multiple alleles (Jönsson, 1972). These alleles may either be situated at different loci or comprise a series of multiple alleles at the same locus. Although this study indicates at least two and possibly three alleles for the control of erucic acid content, Jönsson notes that the occurrence of genes for erucic acid synthesis at still another locus cannot be dismissed.

Higher erucic acid values are found in oil from winter turnip rape than from summer turnip rape. Segregation in the F_1 followed a 1:2:1 distribution indicating that the erucic acid content in winter turnip rape is controlled by a single pair of genes lacking dominance (Jönsson, 1974). Variation in the mean values of erucic acid could not be explained by environmental influence and thus indicates the presence of a series of multiple alleles. It is probable that winter turnip rape has one or more additional alleles than summer turnip rape because of its higher erucic acid levels (Jönsson, 1974).

Although research has led to the widely accepted theory of two gene pairs and a series of multiple alleles for control of erucic acid in rape, and a single gene pair with a series of multiple alleles for turnip rape, the loci have not been identified, their source has not been determined nor has the contribution of alleles controlling erucic acid at each locus been determined.

III. MATERIALS AND METHODS

The plants used in this study consisted of strains from both summer rape and summer turnip rape. Each line was selected for its level of erucic acid in its oil (expressed as a percent of total fatty acids). The rape strains included Target (approximately 40% erucic acid), Bronowski (approximately 11% erucic) and three numbered lines from the University of Manitoba rapeseed breeding program referred to as 339, 5154 and 5318, each with 0%, 48% and 60% erucic acid respectively. The turnip rape strains included the varieties Echo and Polar and a line derived from Yellow Sarson (numbered 9764), with 24%, 28% and 58.5% erucic acid respectively.

A. Development of Monogenic Lines in a Common Background

The monogenic lines developed are homozygous for a single gene pair with each allele contributing from 3 to 12% erucic acid. The monogenic lines were produced by backcrossing lines or varieties with erucic acid in the oil to line 339 with oil essentially free from erucic acid. Assuming the genotype E_oE_o indicates the erucic acid allele derived from B. oleracea and E_cE_c indicates the erucic acid allele derived from turnip rape and e_oe_o or e_ce_c denotes the gene for the absence of erucic acid, the development of monogenic lines in rape can be outlined as follows:

generation 1 : 339 X Target

genotypes : $e_o e_o e_c e_c$ $E_o E_o E_c E_c$

F_1 : $E_o e_o E_c e_c$

backcross : 339 (339 X Target)

BCF_1 : $1E_o e_o E_c e_c : 1E_o e_o e_c e_c : 1e_o e_o E_c e_c : 1e_o e_o e_c e_c$

Select either $E_o e_o e_c e_c$ or $e_o e_o E_c e_c$ using the half-seed technique (Hougen and Bodo, 1973) and self these plants:

$E_o e_o e_c e_c$	$e_o e_o E_c e_c$
X	X
$S_1 : 1E_o E_o e_c e_c$	$S_1 : 1e_o e_o E_c E_c$
$2E_o e_o e_c e_c$	$2e_o e_o E_c e_c$
$1e_o e_o e_c e_c$	$1e_o e_o e_c e_c$

Select either $E_o E_o e_c e_c$ or $e_o e_o E_c E_c$ using the half-seed technique; these are the monogenic lines. This same method was used to obtain monogenic lines from other rape strains.

The isolation of monogenic lines from turnip rape in the rape background can be outlined as follows:

generation 1 : 339 X Echo

genotypes : $e_o e_o e_c e_c$ $E_c E_c$

F_1 : $E_c e_c$

backcross : 339 (339 X Echo)

BCF_1 : $1E_c e_c : 1e_c e_c$

Select $E_c e_c$ using the half-seed technique, and self these plants:

$$E_c e_c$$

X

$$S_1 : 1E_c E_c : 2E_c e_c : 1e_c e_c$$

Select $E_c E_c$, again using the half-seed technique; these plants will be the monogenic lines. The same steps as outlined for Echo were used for Polar and 9764.

The half-seed technique mentioned in both these outlines is a method for oil extraction and fatty acid analysis (Hougen and Bodo, 1973). In this method the outer cotyledon is used for analysis, and the remainder of the embryo is germinated and then planted, thus producing the next generation.

Any one of the lines developed as outlined can be used as a tester to cross with the remaining lines to determine whether the locus in each line is the same or different.

B. Use of Testers to Identify Loci

Two testers derived from the initial cross 339 X Target were chosen for the purpose of obtaining at least one tester with the $E_o E_o$ locus. A third tester derived from the 339 X Echo cross was chosen to represent the $E_c E_c$ locus.

The three testers were planted and all the monogenic lines derived from both rape and turnip rape were crossed with each tester. The seed from these crosses was subsequently planted and the plants selfed. The seed from the selfed plants were analyzed for fatty acid composition and patterns of erucic acid contents were fitted to genetic ratios.

C. Extraction and Analysis of the Oil

Crushed seed was extracted with petroleum ether and the oil simultaneously converted to fatty acid methyl esters by the "rapid methanolysis with sodium" technique described by Hougen and Bodo (1973). The half-seed method for oil extraction and fatty acid analysis (Hougen and Bodo, 1973) was used after the backcross and subsequent selfing generations.

The methyl esters, derived from the outer cotyledon, were analyzed by gas chromatography. Two gas chromatographs, both Varian model 1200, with flame ionization detector, were used with the following conditions:

	Instrument A	Instrument B
Column	OV-1 on Chromosorb W AW DMCS 100-120 mesh (3:97)	as for A
Column length and diameter	8 ft;0.125 o.d. SS	as for A
Oven temperature	230°C	240°C
Injector "	250°C	255°C
Detector "	265°C	265°C
N_2 flow rate	30 ml/min	35 ml/min
Analysis time	4.6 min	5.3 min
Digital electronic integrator	Infotronics model CRS-100	Columbia Scientific Industries model CSI-204

The two instruments, under these conditions, gave approximately 95% agreement on the erucic acid content. However, in order to minimize the error, all analyses which were to be compared to one another were done on the same instrument. The accuracy and precision

of analysis were determined by 20 replicated analyses of a standard seed sample with 5.0% erucic acid in the oil, which gave a mean of 4.9% with a standard deviation of 0.6.

The gas chromatograph column used separated the fatty acids only according to their carbon chain length. Besides the erucic acid, there are other C22 fatty acids (behenic acid, C22:0 and docosadienoic, C22:2) included in the same chromatographic peak. Because their contents are relatively small (less than 1%), they cannot have any appreciable effect on the analytical results.

D. Growth Conditions of the Plants

All plants were grown in the greenhouse where the daylength was regulated to 18 hours per day; the temperature ranged from 20^{o} to $32^{o}C$ in the summer months and remained at approximately $22^{o}C$ in the winter months. To hasten maturity, the lateral racemes were removed and only the terminal raceme was allowed to develop. This did not change the erucic acid content and saved time by hastening maturity (Appendix).

IV. RESULTS AND DISCUSSION

The project consisted of two parts, development of monogenic lines and use of these lines to place the alleles conditioning erucic acid content into the allelic series derived from B. oleracea or the one from turnip rape. Each species is discussed separately.

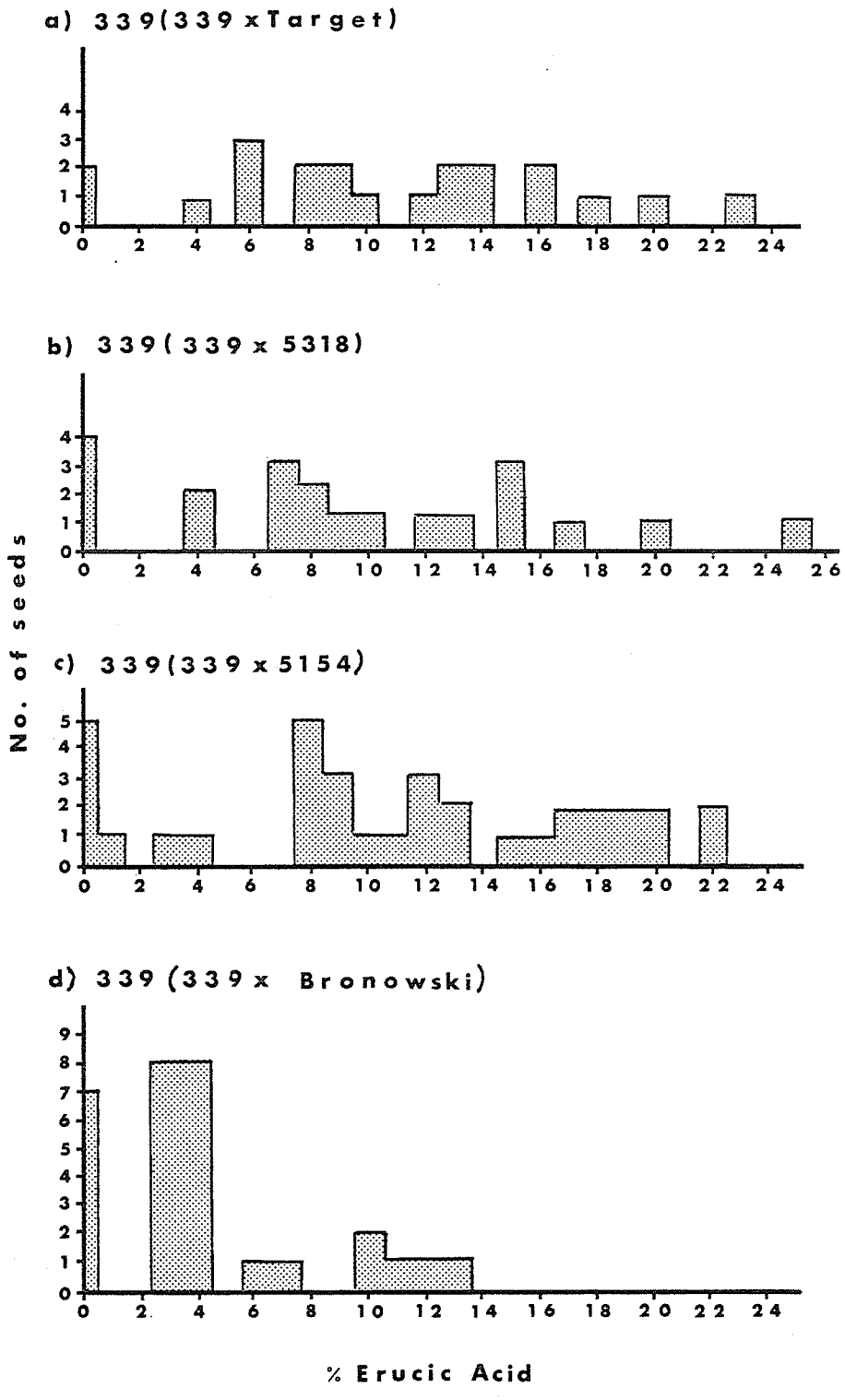
A. Summer Rape: Brassica napus

1. Development of Monogenic Lines

As previously outlined, the rape strains Target, Bronowski, 5318 and 5154 which produced oil containing erucic acid were backcrossed to 339, a strain with seed oil free from erucic acid. Individual seeds from backcrossed plants were analyzed for fatty acid composition using the half-seed technique and erucic acid values were plotted in histograms (Fig. 1). Plants were grown from half-seeds from the middle of the range in each backcross. The selfed seeds from these plants were analyzed for fatty acid content using the single seed technique (Hougen and Bodo, 1973). The erucic acid contents from these seeds were fitted to both a 1:2:1 and a 3:1 ratio.

If the erucic acid content is conditioned by two gene loci the erucic acid content of the individual seeds from the BCF_1 is expected to segregate in a 1:2:1 or a 1:1:1:1 ratio and if only one gene locus is involved a 1:1 ratio is expected. It was difficult to distinguish

Figure 1. Distribution of erucic acid content of oil from F_1 seed from backcrosses involving rape strains a) Target, b) 5318, c) 5154 and d) Bronowski.



between some of the classes with different levels of erucic acid; however, the zero erucic acid class was always distinct. Therefore, the X^2 test for goodness of fit to a 1:2:1 ratio was used for some and the 3:1 for all backcross populations (Table 1). The X^2 test indicated a satisfactory goodness of fit to these ratios for the four backcross populations. Thus, two gene loci were involved in each rape parent which produced oil containing erucic acid. This evidence for two gene loci in Bronowski is in agreement with the report from Jönsson (1977).

TABLE 1. Chi-square values for a 1:2:1 ratio and a 3:1 ratio for rape backcross populations

Backcross	X^2 values for: 1:2:1	3:1
339 (339 X Target)	-	0.06
339 (339 X 5318)	0.60	0.58
339 (339 X 5154)	0.22	1.64
339 (339 X Bronowski)	0.13	0.05

α significant at .05 level

The seeds taken from the middle of the range of erucic contents for each backcross were expected to be of either the genotype $e_o e_o E_c e_c$ or $E_o e_o e_c e_c$. The monohybrid ratios obtained for the individual seeds for all these plants confirmed this expectation (Table 2). The highest class of erucic acid from these plants must therefore have either the $e_o e_o E_c E_c$ or $E_o E_o e_c e_c$ genotype. Progeny from these seeds were taken as monogenic lines.

TABLE 2. Erucic acid contents of selfed seeds from selected plants from the middle of the range of erucic acid content from backcrosses involving rape strains

Selected plants	No. of seeds with percent erucic acid content of					X^2 values for goodness of fit to	
	0 -.5	1.5-5.5	5.5-8.5	8.5-17.5	>20.5	1:2:1	3:1
Target A	6			18	6	1.25	0.47
B	8			18	4	3.60	0.04
C	8			15	6	0.10	0.10
5318 A	6			18	6	1.25	0.47
B	8			18	4	3.60	0.04
C	8			15	6	0.10	0.10
5154 A	9			12	9	1.25	0.25
B	9			12	9	1.25	0.25
C	6			15	9	0.62	0.50
D	8			15	7	0.10	0.04
Bron A	7	13	9			1.14	0.84
B	9	11	7			0.52	0.01
C	5	18	7			1.79	1.50

α significant at .05 level

2. Crosses Between Monogenic Lines and Testers

One locus was transferred from turnip rape into the rape background. This locus was identified as E_cE_c. The tester used for this locus was derived from Echo. The other tester must contain the E_oE_o locus. This second tester could not be identified until after the crosses between the lines had been made, the progeny selfed and the seeds analyzed. Selection of two lines derived from Target increased the probability of including the E_oE_o locus.

Segregation patterns indicated that the loci from the monogenic lines from Target were the same and different from the line from Echo (Table 5). Therefore, Target-12 was chosen as a tester and labelled T_1 and Echo-18 was labelled T_2.

No segregation would be expected from crosses between lines or testers with erucic acid controlled by the same locus if the contribution from both alleles is equal. If the allelic contribution differed there would be limited segregation in monohybrid ratio.

The selfed progeny of crosses of testers or lines with different loci segregate in dihybrid ratios and zero erucic acid levels should be recovered. Thus, it is only necessary to distinguish between mono- or dihybrid ratios to determine if the alleles in the monogenic lines are on the same or different loci.

The dihybrid ratios vary according to the allelic contribution from each line. Equal contribution of alleles would result in a 1:4:6:4:1 dihybrid ratio. As alleles do not always have the same dosage effect this ratio could fall into seven or nine phenotypic classes depending on whether the allelic contribution was approximately 2:1 or more unequal (Table 3).

TABLE 3. Expected phenotypes in F_2 from crosses involving two different loci conditioning erucic acid content with equal and with unequal phenotypic expression for the alleles

	Phenotypic expression of alleles		
	$e_o=e_o=0$	$e_o=e_c=0$	$e_o=e_c=0$
	$E_o=E_c=10$	$E_o=10;E_c=5$	$E_o=10;e_c=3$
F_2 genotypes	Phenotypes (expressed as % erucic acid)		
$E_oE_oE_cE_c$	40	30	26
$E_oE_oE_ce_c$	30	25	23
$E_oe_oE_cE_c$	30	20	16
$E_oE_oe_ce_c$	20	20	20
$E_oe_oE_ce_c$	20	15	13
$e_oe_oE_cE_c$	20	10	6
$E_oe_ce_ce_c$	10	10	10
$e_oe_cE_ce_c$	10	5	3
$e_oe_oe_ce_c$	0	0	0

The monogenic lines derived from rape varied in erucic acid content from 6 to 21% (Table 4). Therefore a 1:4:6:4:1 ratio would not be expected in every case. This makes classification of the data difficult, and overlap of classes makes the 1:4:11 or 1:3:12 or 1:2:13 ratios a more reasonable test for goodness of fit.

The erucic acid values for oil from F_2 seeds from crosses between tester 2, with a gene pair from Echo, and five monogenic lines derived from Target segregated in the manner expected for a dihybrid (Table 5); thus different loci were involved. The erucic acid values from crosses involving tester 1, with a gene pair from Target, varied in a limited manner. This indicated that the same locus was involved in the five lines derived from Target (Table 5). Since the locus in tester 2 was derived from B. campestris it was designated E_c and as the locus in tester 1 was different and probably derived from B. oleracea it was designated E_o. Thus, the evidence indicates that all Target lines contained the E_oE_o locus.

The results with Targ-11 originally indicated a spread of 7 to 13% erucic for the F_2 from T_1 X Targ-11 and there were only three discrete classes isolated in the F_2 of T_2 X Targ-11. As these results were peculiar, another set of seeds was analyzed. The new set conformed closely to the expected ratios (Table 5). One possible explanation for the original results is that the seeds were immature. The erucic acid content of the seed fatty acids are known to increase with maturation of the seed; therefore, more mature seeds should have a higher erucic acid content and thus the classes would be more evident. Diseases could also influence the fatty acid composition by reducing the nutrients available for the developing seed.

TABLE 4. Erucic acid levels for monogenic lines derived from rape and turnip rape

Source of line	Name of line	% erucic acid
Rape:		
Target	Targ-15	18.0
	Targ-7	19.0
	Targ-11	20.0
	Targ-8	18.5
	T_1(Targ-12)	20.6
5318	5318-2	23.0
	5318-4	15.0
5154	5154-21	21.0
	5154-16	22.0
	5154-13	12.0
	5154-1	12.0
Bronowski	Bron-2	7.5
	Bron-5	7.5
	Bron-1	6.0
Turnip rape:		
Echo	T_2(Echo-18)	16.0
	Echo-24	21.0
Polar	Polar-12	15.0
	Polar-16	20.0
9764	9764-3	22.0
	9764-1	20.0
	9764-5	24.0
	9764-2	22.0

TABLE 5. Erucic acid distribution and chi-squares for individual F_2 seeds from crosses between rape monogenic lines derived from Target and two testers used to identify the locus in each line

Cross		X^2 value for 1:4:6:4:1	X^2 value for 15:1	X^2 value for 1:4:11	Locus identified
T_1 X T_1					
Observed classes[α]	14-19				E_oE_o
No. of seeds[β]	32				
T_2 X T_1					
Observed classes	0;9-14;17-23;25-27;31-33				
No. of seeds	1:8:15:5:3	3.7	1.0	1.0	E_oE_o
T_1 X Targ-7					
Observed classes	14-21				E_oE_o
No. of seeds	32				
T_2 X Targ-7					
Observed classes	0;8-10;16-21;26-30;35				
No. of seeds	1:10:14:6:1	3.3	1.0	1.0	E_oE_o
T_1 X Targ-8					
Observed classes	15-28				
No. of seeds	16				E_oE_o
T_2 X Targ-8					
Observed classes	0;8-9;18-20;22-28;30-32				
No. of seeds	1:4:4:5:2	1.7	0.0	0.0	E_oE_o
T_1 X Targ-11					
Observed classes	10-27				
No. of seeds	32				E_oE_o
T_2 X Targ-11					
Observed classes	0;6-11;15-20;22-27;31-34				
No. of seeds	1:7:11:11:2	1.3	1.0	1.3	E_oE_o
T_1 X Targ-15					
Observed classes	15-34				
No. of seeds	32				E_oE_o
T_2 X Targ-15					
Observed classes	7-10;14-25;27-30;33				
No. of seeds	0:6:21:4:1	9.9[γ]	9.9[γ]	9.9[γ]	E_oE_o

α expressed by erucic acid content (% of total fatty acids)
β F_2 seeds per class
γ significant at .05 level

While the data for the F_2 from T_2 X Targ-15 suggest a dihybrid ratio, no seeds were recovered in the zero class and the X^2 test did not indicate a satisfactory fit to the expected dihybrid ratios. However, the segregation from the F_2 of T_1 X Targ-15 was limited indicating that the E_oE_o locus is involved in the control of erucic acid inheritance.

The line 5318-4 segregated in the same manner as the lines from Target. There were no data from 5318-2 as all plants were damped-off as seedlings. The E_oE_o locus was identified in line 5318-4 (Table 6).

Both the E_oE_o and E_cE_c loci were identified in lines from 5154. Due to the unequal contribution of alleles in crosses with 5154-1, 5154-13 and the testers, the results could not be expected to fit a 1:4:6:4:1 ratio. However, the erucic acid values in the F_2 from crosses with tester 1 show limited segregation in both cases. The F_2 from 5154-1 and 5154-13 crosses with tester 2 fit a 1:4:11 ratio and thus the E_oE_o locus was identified in these lines (Table 6). The F_2 from crosses involving lines 5154-16 and 5154-21 would be expected to segregate in a 1:4:6:4:1 ratio as both these lines have approximately the same erucic acid content as tester 1. The data from the lines do not segregate according to this ratio, however, as both lines fit the 1:4:11 ratio and as there is limited segregation in crosses with T_2 the E_cE_c locus was identified (Table 6).

The contribution of the alleles in the lines derived from Bronowski was much lower than the allelic contribution from the testers (Table 4). The F_2 data from Bron-1 and Bron-5 fits a 1:4:11 ratio in crosses with T_1; the F_2 data from T_1 X Bron-2 does not fit the dihybrid ratio.

TABLE 6. Erucic acid distribution and chi-squares for individual F_2 seeds from crosses between rape monogenic lines derived from 5318 and 5154 and two testers used to identify the locus in each line

Cross		X^2 values for 1:4:6:4:1	X^2 values for 15:1	X^2 values for 1:4:11	Locus identified
T_1 X 5318-4					
Observed classes[α]	16-30				
No. of seeds[β]	32				E_oE_o
T_2 X 5318-4					
Observed classes	0;8-15;18-40				
No. of seeds	3:12:17	-	3.4	0.4	E_oE_o
T_1 X 5154-1					
Observed classes	13-24				
No. of seeds	32				E_oE_o
T_2 X 5154-1					
Observed classes	0;5-11;15-21;23-27				
No. of seeds	2:9:12:9:0	9.9[γ]	0.0	0.2	E_oE_o
T_1 X 5154-13					
Observed classes	13-26				
No. of seeds	32				E_oE_o
T_2 X 5154-13					
Observed classes	0;6-11;16-26;31				
No. of seeds	3:12:16:1	-	0.4	3.1	E_oE_o
T_1 X 5154-16					
Observed classes	0;6-14;18-24;26-31				
No. of seeds	1:6:11:14:0	-	1.0	2.0	E_cE_c
T_2 X 5154-16					
Observed classes	12-25				
No. of seeds	32				E_cE_c
T_1 X 5154-21					
Observed classes	0;8-10;14-20;23-29;31-34				
No. of seeds	2:3:14:10:8	9.3	0.0	9.3[γ]	E_cE_c
T_2 X 5154-21					
Observed classes	17-23				
No. of seeds	32				E_cE_c

α expressed by erucic acid content (% of total fatty acids)
β F_2 seed per class
γ significant at .05 level

The data from all the lines show limited segregation in F_2 data from crosses with T_2. Thus, the data for all lines indicates that the E_cE_c locus is involved in erucic acid control. Because of the unequal contribution of the alleles F_2 data from both T_2 X Bron-1 and T_2 X Bron-2 show a good fit to a 1:2:1 ratio while the F_2 data from T_2 X Bron-5 fit a 3:1 ratio. The homozygous Bronowski alleles contribute 5 to 8% erucic; 10 to 15% erucic represents the heterozygous condition and 17 to 33% erucic the homozygous tester alleles (Table 7).

The results of the rape lines tested indicate that two distinct loci are involved in the inheritance of erucic acid in rape. More than one allele was identified on each locus. Alleles contributing 6, 7.5 and 9 to 10% erucic acid were identified on the E_oE_o locus. Alleles contributing 3 to 4% and 9 to 10% were identified on the E_cE_c locus. As the plants were grown in the greenhouse the erucic acid levels are probably lower than would be obtained if the plants were grown in the field.

The monogenic lines were developed in a manner that ensures that each line contains a single gene pair conditioning the production of erucic acid. Limited segregation in the F_2 from a cross between a line and a tester indicates that the same locus is involved in both the line and the tester. Any approach to a dihybrid ratio indicates that different loci are involved in the tester and the line. Therefore, deviations from expected ratios can be attributed to other causes such as a low number of individuals per sample, difficulty in placing individuals in the proper classes and the statistical probability that some of the data may not appear to fit a ratio due to chance alone.

TABLE 7. Erucic acid distribution and chi-squares for individual F_2 seeds from crosses between rape monogenic lines derived from Bronowski and two testers used to identify the locus in each line

Cross		X^2 values for 1:4:6:4:1	X^2 values for 15:1	X^2 values for 1:4:11	Locus identified
T_1 X Bron-1					
Observed classes[α]	0-2;5-10;12-16;18-20;23-26				
No. of seeds[β]	2:8:14:3:5	10.4[γ]	0.0	0.0	E_cE_c
T_2 X Bron-1					
Observed classes	5-8;10-15;17-20				
No. of seeds	9:14:9	X^2 for 1:2:1 = 0.20			E_cE_c
T_1 X Bron-2					
Observed classes	0-2;12-25;28-32;35				
No. of seeds	4:20:7:1	-	1.1	26.3[γ]	E_cE_c
T_2 X Bron-2					
Observed classes	5-8;10-22;24-33				
No. of seeds	10:15:7	X^2 for 1:2:1 = 0.08			E_cE_c
T_1 X Bron-5					
Observed classes	0;2-5;7-16;20;32				
No. of seeds	3:6:21:1:1	54.8[γ]	0.40	1.0	E_cE_c
T_2 X Bron-5					
Observed classes	5-6;10-17				
No. of seeds	7:25	X^2 for 3:1 = 0.2			E_cE_c

α expressed by erucic acid content (% of total fatty acids)
β F_2 seeds per class
γ significant at .05 level

Thus, several alleles were identified on the two loci and there was no evidence for a third locus in any of the monogenic lines tested.

B. Summer Turnip Rape: Brassica campestris

1. Development of Monogenic Lines

The summer turnip rape strains used were Echo, Polar and a line isolated from Yellow Sarson numbered 9764. The strains were backcrossed to 339, the rape strain with seed oil free from erucic acid. Thus, all the monogenic lines were developed in a common background.

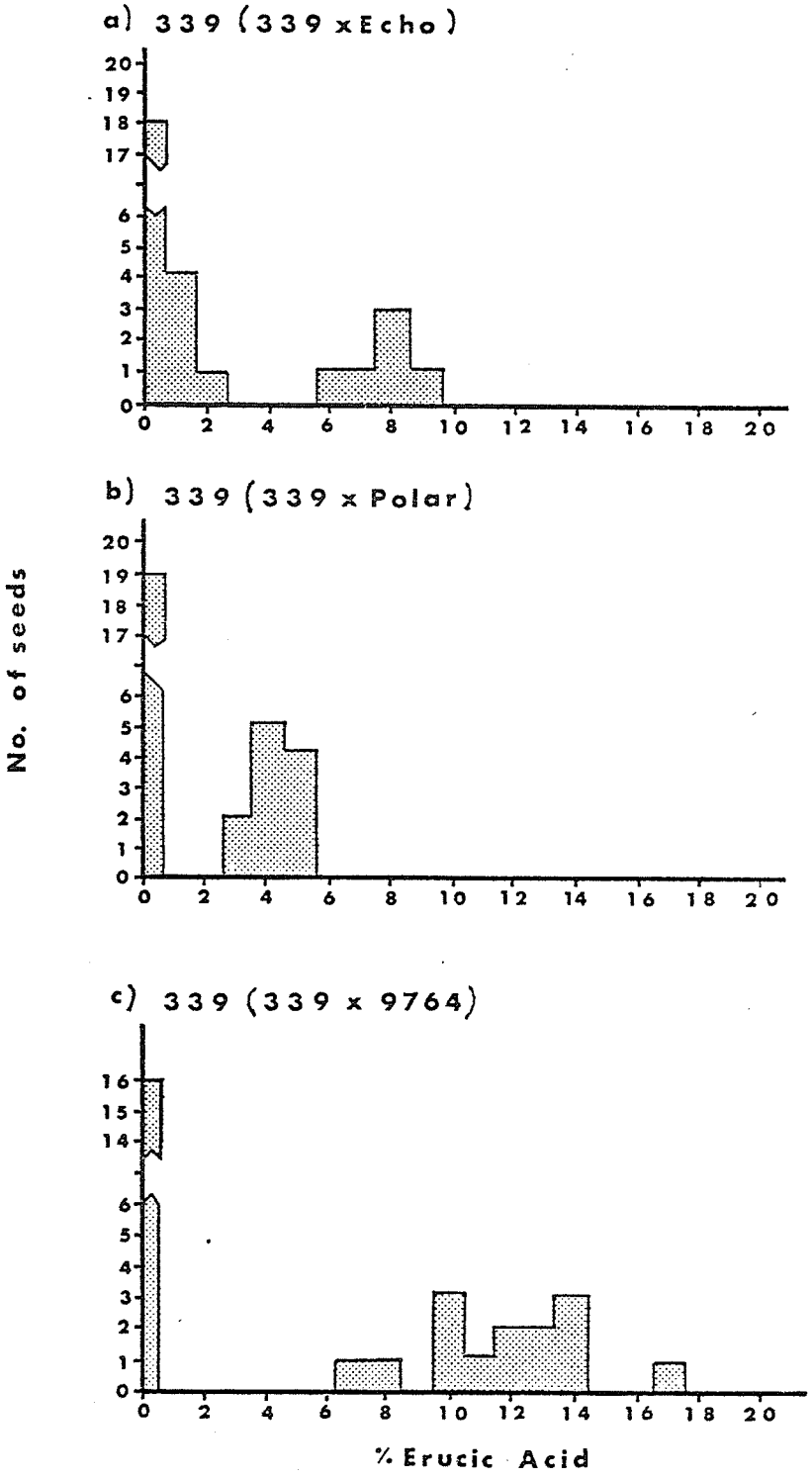
Only one locus is expected to condition erucic acid in summer turnip rape; therefore, the erucic acid content of the individual seed from the BCF_1 is expected to segregate in a 1:1 ratio. The erucic acid values from each backcross were plotted in histograms (Fig. 2). Echo, Polar and 9764 fit the expected ratio (Table 8). However, the erucic acid contents in the Echo backcross fell into two distinct classes (Fig. 2a).

TABLE 8. Chi-square values for a 1:1 ratio for the turnip rape backcross populations

Backcross	X^2 values for 1:1
339 (339 X Echo)	1.7
339 (339 X Polar)	2.3
339 (339 X 9764)	0.1

α significant at .05 level

Figure 2. Distribution of erucic acid content of oil from F_1 seed from backcrosses involving turnip rape strains a) Echo, b) Polar and c) 9764.



Echo is cross-pollinated and the variety may contain several alleles for erucic acid. The data from the Echo backcross suggest that Echo was heterozygous for alleles conditioning erucic acid. Its genotype may be $E_{c_1}E_{c_2}$ with the contribution of one allele for erucic acid production much greater than that of the other.

The erucic acid content of the non-zero class in the backcrosses with Echo, Polar and 9764 is noticeably lower than would normally be expected. The non-zero class would be expected to range around 12% erucic in Echo, 14% erucic in Polar and 28% erucic in 9764. The actual results were 5.5 to 9.5%, 2.5 to 5.5% and 6.5 to 17.5%, respectively. This deviation could be due to a dilution effect when genes from turnip rape are transferred to rape. Approximately half of the oil synthesized in rape should come from each genome and as the B. oleracea genome in the rape strain 339 did not have genes for erucic acid production the content of erucic acid should be approximately half of that expected in turnip rape.

The distribution of the erucic acid contents of individual seeds from seven of the nine plants selected from the BCF_1 populations were tested for goodness of fit to monohybrid (3:1 and 1:2:1) ratios (Table 9). Chi-square tests indicated a satisfactory fit. Due to poor seed set only eight seeds were obtained from the plant Polar A. The distribution of erucic acid contents in oil from individual seeds from this plant appears to represent monohybrid segregation and fits a 3:1 ratio satisfactorily.

The distribution of erucic acid contents of seed from Echo A did not fit either of the expected monohybrid ratios satisfactorily (Table 7)

TABLE 9. Erucic acid contents of selfed seed from selected plants from the high range of erucic acid content from each backcross with turnip rape strains

Selected plants	No. of seeds with percent erucic acid content of			X^2 values for goodness of fit to	
	0-.5	6-7	over 18	1:2:1	3:1
Echo A	3	15	11	7.31^{α}	$6.76^{\alpha\beta}$
B	6	10	4	0.42	0.24
Polar A	1	3	4	-	1.14
B	8	9	8	2.09	0.56
9764 A	4	17	9	3.55	3.06
B	10	15	5	1.88	0.94
C	5	15	8	0.99	0.97
D	5	15	10	1.56	1.50
E	9	13	8	0.59	0.36

α significant at .05 level
β significant at .01 level

and it does not fit a 15:1 ratio satisfactorily ($X^2 = 8.0$, $p < .01$). The distribution of erucic acid content from individual seeds from Echo A could have been affected by several factors such as partial sterility, aneuploidy (which occurs in crosses between rape and turnip rape) and the small size of the sample. Since Echo A was derived from a half-seed from the high erucic class in the backcross generation (Fig. 2a), its genotype should have been $E_c e_c$. Thus, the distribution of erucic acid contents from Echo A (Table 9) probably represents a disturbed monohybrid ratio.

Some deviations from monohybrid ratios have been reported for lines derived from Yellow Sarson (Dorrell and Downey, 1964). The authors

suggested that taxonomic differences between turnip rape and Yellow Sarson could account for these deviations. Similar deviations were not encountered in this study. The erucic acid contents of individual seeds from the five BCF_1 plants grown from half-seeds from the upper range of erucic contents from 9764 (Fig. 2c) all gave satisfactory agreement with monohybrid ratios (Table 9).

The genotypes of the low, intermediate and high erucic acid classes (Table 9) all apparently represent monohybrid segregation and should be e_ce_c, E_ce_c and E_cE_c, respectively. Plants were grown from half-seeds from the high erucic acid class (E_cE_c) and used as monogenic lines.

2. Crosses Between Monogenic Lines and Testers

The distribution of erucic acid contents for individual seeds from F_1 plants (i.e., F_2 seeds) from crosses of seven of the eight monogenic lines derived from turnip rape to tester 1 approximated dihybrid ratios. Chi-square tests indicated agreement with dihybrid ratios (Tables 10 and 11). This dihybrid segregation, which included recovery of the zero erucic acid class, indicates that the genes conditioning erucic acid content in tester 1 and in these monogenic lines are on different loci. The segregation of erucic acid content from the F_2 of crosses involving tester 2 and all eight monogenic lines was limited to a narrow range. This lack of segregation, or limited segregation without recovery of the zero erucic acid class, indicates that the locus in tester 2 and in all eight monogenic lines is the same (Tables 10 and 11).

The distribution of erucic acid content in F_2 seeds from the cross T_1 X 9764-1 was tested for goodness of fit to dihybrid ratios and X^2 tests indicated that the fit was not satisfactory. However, segregation

TABLE 10. Erucic acid distribution and chi-squares for individual F_2 seeds from crosses between turnip rape monogenic lines derived from Echo and Polar and two testers used to identify the locus in each line

Cross		X^2 values for 1:4:6:4:1	X^2 values for 15:1	X^2 values for 1:4:11	Locus identified
T_1 X T_2 (Echo-18)					
Observed classes[α]	0;6-10;12-21;24-27;29-30				
No. of seeds[β]	2:5:16:5:4	5.6	1.1	2.8	E_cE_c
T_2 X T_2					
Observed classes	14-26				
No. of seeds	32				E_cE_c
T_1 X Echo-24					
Observed classes	0;9-13;18-25;29-34;38-39				
No. of seeds	2:11:10:5:2	3.0	0.0	2.3	E_cE_c
T_2 X Echo-24					
Observed classes	18-25				
No. of seeds	32				E_cE_c
T_1 X Polar-12					
Observed classes	0;5-12;14-23;26-32				
No. of seeds	3:8:20	-	0.4	0.4	E_cE_c
T_2 X Polar-12					
Observed classes	10-24				
No. of seeds	32				E_cE_c
T_1 X Polar-16					
Observed classes	0;5-9;13-22;25-29;36				
No. of seeds	1:5:16:8:1	2.0	1.0	3.2	E_cE_c
T_2 X Polar-16					
Observed classes	9-25				
No. of seeds	32				E_cE_c

α expressed by erucic acid content (% of total fatty acids)
β F_2 seeds per class
γ significant at .05 level

TABLE 11. Erucic acid distribution and chi-squares for individual F_2 seeds from crosses between turnip rape monogenic lines derived from 9764 and two testers used to identify the loci involved

Cross		X^2 values for 1:4:6:4:1	X^2 values for 15:1	X^2 values for 1:4:11	Locus identified
T_1 X 9764-1					
Observed classes[α]	8-12;15-21;23-29;32				
No. of seeds[β]	9:16:16:1	9.9[γ]	9.9[γ]	9.9[γ]	E_cE_c
T_2 X 9764-1					
Observed classes	11-27				
No. of seeds	32				E_cE_c
T_1 X 9764-2					
Observed classes	0;7-10;17-25;27-29;40				
No. of seeds	4:5:17:5:1	7.1	1.1	3.4	E_cE_c
T_2 X 9764-2					
Observed classes	17-31				
No. of seeds	32				E_cE_c
T_1 X 9764-3					
Observed classes	0;8-13;16-22;24-29;33				
No. of seeds	1:7:13:9:2	1.3	1.0	1.3	E_cE_c
T_2 X 9764-3					
Observed classes	17-29				
No. of seeds	32				E_cE_c
T_1 X 9764-5					
Observed classes	0;7-12;16-24;26-32;37-38				
No. of seeds	1:6:13:9:2	1.9	1.0	2.0	E_cE_c
T_2 X 9764-5					
Observed classes	15-29				
No. of seeds	32				E_cE_c

α expressed by erucic acid content (% of total fatty acids)
β F_2 seeds per class
γ significant at .05 level

of erucic acid from F_2 seeds from the cross with tester 2 was limited indicating that the same locus (E_cE_c) was involved in T_2 and monogenic line 9764-1. For this reason the distribution of erucic acid in F_2 seed from the cross T_1 X 9764-1 can be assumed to be a disturbed dihybrid ratio.

Thus, the alleles conditioning erucic acid in monogenic lines derived from turnip rape are all on one locus, the locus derived from turnip rape. A series of alleles conditioning the levels of erucic acid content were identified on this locus. These alleles contributed 3 to 4%, 7 to 8% and 10 to 12% erucic acid. As these alleles were transferred into the amphidiploid rape background the levels of expression appear to be considerably lower than in the diploid background of turnip rape. Since the number of strains of turnip rape used in this experiment was limited, it is likely that more alleles are present in other varieties or strains at this locus.

Testers for a locus from turnip rape (E_cE_c) and for a locus from B. oleracea (E_oE_o) have been established in rape. These testers could be used with other monogenic lines derived from rape or turnip rape in an attempt to find a third locus conditioning the inheritance of erucic acid.

V. SUMMARY AND CONCLUSIONS

Published reports indicate erucic acid content in seed oil from turnip rape is controlled by a single gene pair with the genes acting in an additive manner. Several reports indicate that two gene pairs, with genes acting in an additive manner, control erucic acid content in rape. Since rape is an amphidiploid made up of a genome from turnip rape and one from B. oleracea and the seed oils from both these species contain erucic acid, each species apparently contributed at least one gene pair to rape.

Monogenic lines with genes for erucic acid production were developed from rape and turnip rape. A monogenic line with a gene pair from turnip rape (E_cE_c) was used to identify a monogenic tester for the locus (E_oE_o) derived from B. oleracea. The alleles in all the rape lines with genes for erucic acid derived from turnip rape were all on the same locus (E_cE_c). Monogenic lines with genes conditioning erucic acid content on two loci (E_cE_c and E_oE_o) were derived from rape. Allelic series conditioning different levels of erucic acid content were found for both loci.

Several authors have postulated that more than two loci might be involved in the control of erucic acid content in the seed oil from rape. Sources of genes representing a wide range of erucic acid levels in rape and turnip rape were used in the development of the monogenic lines used in this study. Nevertheless, no evidence for a third locus

conditioning erucic acid was found. While multiple alleles at two loci provide an adequate explanation of most of the data now available, the testers for the two loci are available for experiments designed to discover whether other loci are involved in other strains of rape and turnip rape.

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VII. APPENDIX

The time required to develop mature seeds from both rape and turnip rape can be shortened by allowing only the terminal raceme of the plant to develop. Such a procedure would be useful to reduce the time required for an experiment; however, the validity of the results might be questioned. Removal of the lateral racemes increases the nutrients available for the main raceme and thus there is an increase in the growth of remaining fruits. This change might influence the composition of the seed oil. The following study was undertaken to determine whether removal of the lateral racemes would change the fatty acid composition of the seed from the main raceme.

Three lines of rape were selected from those used in the genetic study and grown under greenhouse conditions; the first had a relatively high content of erucic acid (about 21.5%), the second, a content of 5% erucic and the third, a content of 0.3% erucic. Forty plants of each line were grown. Of these, 20 plants were taken at random and only the terminal raceme was allowed to develop. The remaining 20 plants were allowed to develop the terminal raceme and five other racemes. At harvest, the siliques were divided for analysis into three groups for each line. The first was the group of 20 plants which had only the terminal raceme develop. The second included the terminal racemes of the plants which were allowed to branch and the third included the lower racemes of these same plants.

Samples used for analysis consisted of approximately 0.25 g of seed. The analyses were conducted as described in "Materials and Methods" with one exception. The gas chromatographic column used here was 3% SP2310 with 2% SP2300 on 100 to 120 mesh Chromosorb W AW.

To test the hypothesis that the means of the different fatty acids were the same among the three different groups a one way analysis of variance for equal sample size was carried out for the high and low erucic lines. As one plant in the middle line did not set seed a one way analysis of variance for unequal sample size was used.

There was no significant difference (at the .05 level) in the fatty acid composition within the high and middle lines (Tables 1, 2 and 3). However, the low line does show a significant difference in the means of both the oleic and linoleic acid contents. As these plants were all grown in the same environment, the differences cannot be attributed to changes in the environment. However, in none of the lines was there a significant difference in the contents of any of the longer chain fatty acids.

These data indicate that the technique of hastening maturity is valid for experiments dealing with the longer chain fatty acids.

TABLE 1. A comparison of the means of the fatty acid percentage for the high line

Fatty acid	Means for plants with only terminal raceme	Means for the terminal racemes of branched plants	Means for lateral racemes	F(2,57)
Palmitic (C16:0)	3.9	3.7	3.9	2.46
Oleic (C18:1)	32.9	32.0	32.0	0.38
Linoleic (C18:2)	15.6	15.1	15.3	0.25
Linolenic (C18:3)	7.1	7.2	7.1	0.04
Eicosenoic (C20:1)	14.6	15.6	15.6	5.74
Erucic (C22:1)	21.3	21.9	21.7	0.66

α significant at .05 level

TABLE 2. A comparison of the means for the fatty acid percentages for the middle line

Fatty acid	Means for plants with only terminal raceme	Means for the terminal racemes of branched plants	Means for lateral racemes	F(2,55)
Palmitic (C16:0)	2.7	2.5	2.9	2.01
Oleic (C18:1)	57.6	57.3	56.0	0.59
Linoleic (C18:2)	14.6	15.2	14.7	0.64
Linolenic (C18:3)	6.7	6.5	6.8	0.12
Eicosenoic (C20:1)	10.2	10.6	11.3	2.68
Erucic (C22:1)	5.1	4.9	4.8	1.21

α significant at .05 level

TABLE 3. A comparison of means of the fatty acid percentages for the low line

Fatty acid	Means for plants with only terminal raceme	Means for the terminal racemes of branched plants	Means for lateral racemes	F(2,57)
Palmitic (C16:0)	4.4	3.4	3.1	5.26
Oleic (C18:1)	65.3	70.6	68.3	8.45$^{\alpha}$
Linoleic (C18:2)	17.6	15.2	16.7	15.51$^{\alpha}$
Linolenic (C18:3)	7.9	6.7	7.3	3.06
Eicosenoic (C20:1)	0.7	0.3	0.5	1.67
Erucic (C22:1)	0.6	0.2	0.1	3.47

α significant at .05 level