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Genetic control of polyunsaturated fatty acid biosynthesis in flax (*Linum usitatissimum*) seed oil

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Summary. The inheritance of two mutants of flax (*Linum usitatissimum*), having altered proportions of the C18 polyunsaturated fatty acids, linoleic and linolenic, was examined. Both lines, 'M1589' and 'M1722', are homozygous for a single gene mutation which reduces linolenic acid content from 34% to 22% and raises linoleic acid from 15% to 27%. Genetic analysis of crosses involving 'M1589', 'M1722' and their parental cultivar 'Glenelg' revealed that these mutations are in different unlinked genes and exhibit additive (codominant) gene action. The symbols *Ln1* and *Ln2* are proposed for the mutated genes in 'M1589' and 'M1722', respectively. Recombinant genotypes homozygous for the mutant alleles at both loci are very low in linolenic acid (2%) and high in linoleic acid (48%), with unaltered proportions of other fatty acids. The complete inverse correlation between linoleic and linolenic acids ($r = -0.98$) indicates that the mutations block the synthesis of linolenic acid at the linoleic desaturation step.

Key words: Flax – Linseed oil – Linoleic acid – Linolenic acid – Fatty acid desaturation

Introduction

Seed storage lipids are the major source of edible vegetable oils, the quality of which is dependent primarily on their fatty acid composition. Of particular importance is the relative proportion of the C18 unsaturated fatty acids namely oleic acid (C18 : 1), linoleic acid (C18 : 2) and linolenic acid (C18 : 3). These fatty acids determine the end use of the oil, those being high in linoleic acid, e.g. sunflower and safflower, being utilised in polyunsaturated oils and margarines, while those that are high in oleic acid content are more suited to cooking and salad oils e.g. peanut and rapeseed. Oils having high levels of linolenic acid are unsuitable for use in edible products due to problems of flavour reversion associated with the autoxidation of this unstable fatty acid (Smouse 1979).

The relatively minor chemical changes required to interconvert individual fatty acids, combined with the apparent lack of functional constraints on seed storage lipid composition (Röbbelen 1982), have enabled this character to be readily manipulated in several oilseed species. However, it has not proved possible to eliminate linolenic acid from vegetable oils, such as soybean and rapeseed, that contain appreciable levels of this undesirable fatty acid, although breeders have made some progress towards reducing its content in both species through mutation breeding (Röbbelen and Nitsch 1975; Wilcox et al. 1984). The seed oil of flax (*Linum usitatissimum*), known as linseed oil, normally contains between 45% and 65% linolenic acid, a level which has precluded its use as an edible oil and given the oil its traditional industrial usage. Recently, however, a mutant genotype of flax containing less than 2% linolenic acid has been isolated (Green 1986). This modification was achieved by selection within the F_2 generation of a cross between 'M1589' and 'M1722', two induced mutants (Green and Marshall 1984) with reduced levels of linolenic acid (28–30%). This virtual elimination of linolenic acid from the seed lipids is accompanied by an equivalent increase in the content of linoleic acid, the proportions of other fatty acids remaining unchanged. These changes indicate that the mutations block the final desaturation of linoleic to linolenic acid.

This paper reports the genetic analysis of the 'M1589' and 'M1722' mutants and considers their implications for the biosynthesis of polyunsaturated fatty acids in the seed lipids of *L. usitatissimum*.

Materials and methods

The parental lines used in this analysis consisted of the cultivar 'Glenelg' and two M_4 plants, 'M1589' and 'M1722',

derived from 'Glenelg' by EMS (ethyl methanesulphonate) – mutagenesis and apparently homozygous for the mutations. The crosses analysed were 'Glenelg'×'M1589', 'M1722'× 'Glenelg' and 'M1722'×'M1589'. Single plants were used as parents in all crosses to produce F_1 and backcross populations. Additionally, an F_2 population from the cross 'M1722'× 'M1589' was examined.

Individual crosses were grown in separate experiments as outlined below (population sizes shown in brackets):

Experiment 1: 'Glenelg'×'M1722'
'Glenelg' (5), 'M1722' (5), F_1 (4),
F_1×'Glenelg' (10), F_1×'M1722' (15).

Experiment 2: 'M1589'×'Glenelg'
'Glenelg' (6), 'M1589' (6), F_1 (6),
F_1×'Glenelg' (20), F_1×'M1589' (20).

Experiment 3: 'M1722'×'M1589'
'M1722' (5), 'M1589' (5), F_1 (3),
F_1×'M1722' (27), F_1×'M1589' (27).

Experiment 4: 'M1722'×'M1589'
F_2 (114)

The occurrence of several 'M1722'×'M1589' F_2 plants having very low levels of linolenic acid (less than 2%) indicated that the 'M1589' and 'M1722' mutations were in different genes. Therefore, a further experiment was conducted to determine the fatty acid composition of all possible genotypes at the two loci. In this experiment (Experiment 5) 'Glenelg', 'M1589', 'M1722' and an 'M1722'×'M1589' F_2 plant (homozygous for both mutations and hereafter referred to as 'Zero') were crossed in a half-diallel design; that is, in all possible combinations excluding reciprocals. Parental and F_1 genotypes (6 plants per genotype) were grown together under the same greenhouse conditions as in Experiments 1 to 4.

Experiments were conducted in a greenhouse under natural photoperiod but with temperature controlled at 27 °C (12-h-day)/20 °C (12-h-night). Plants were harvested at maturity and a 30-seed sample taken for analysis of fatty acid composition. Samples were crushed between filter paper discs to extrude the oil and transmethylated in 2% methanolic sulphuric acid using the method of Welch (1977). Fatty acid methyl esters prepared in this way were analysed by standard gas chromatography procedures (Green and Marshall 1981).

Fatty acid composition data are presented as percentages of total fatty acid content or expressed as the parameters ODP (oleic desaturation proportion) and LDP (linoleic desaturation proportion), derived by the following formulae:

$$ODP = (\%\ \text{linoleic} + \%\ \text{linolenic}) \div (\%\ \text{oleic} + \%\ \text{linoleic} + \%\ \text{linolenic})$$

$$LDP = \%\ \text{linolenic} \div (\%\ \text{linoleic} + \%\ \text{linolenic})$$

The magnitudes of these parameters are directly proportional to the activities of the individual enzyme systems (Cherif et al. 1975) believed to be responsible for the desaturation of oleic acid and linoleic acid respectively.

Means were calculated for all characters in the parental and F_1 generations and compared using the 't' test. Backcross and F_2 plants were assigned to phenotypic classes based on the appearance of discontinuities in the frequency distributions. The proportions of plants observed in each phenotypic class were compared to those expected on the basis of appropriate genetic hypotheses. Goodness of fit to tested ratios was measured by the chi-squared statistic.

Results

Experiments 1 and 2

The results for fatty acid composition in Experiments 1 and 2 are presented in Table 1. The performance of the cultivar 'Glenelg', which was common to both experiments, indicates that the environmental conditions during Experiment 1 resulted in a more unsaturated fatty acid composition. This was due to a higher level of oleic acid desaturation, as evidenced by the higher ODP in Experiment 1, the level of linoleic desaturation (LDP) not being significantly different between experiments.

Within experiments, 'M1589' and 'M1722' did not differ from 'Glenelg' in oleic acid content or ODP. Small differences were evident for some components, 'M1722' having slightly higher contents of palmitic and stearic acids than 'Glenelg'. However, in both experi-

Table 1. Means and mid-parent values for fatty acid composition in the parental and F_1 generations of the crosses 'M1589'×'Glenelg' and 'Glenelg'×'M1722'

Genotype	Fatty acid composition (%)					ODP	LDP
	16:0	18:0	18:1	18:2	18:3		
Experiment 1							
'Glenelg'	7.0[a]	3.7[a]	35.1[a]	14.1[a]	40.1[a]	0.61[a]	0.74[a]
'M1722'	7.4[b]	4.4[b]	35.1[a]	27.2[b]	25.7[b]	0.60[a]	0.49[b]
F_1	7.4[b]	4.2[b]	35.7[a]	20.9[c]	31.7[c]	0.60[a]	0.60[c]
MP value	7.2	4.1	–	20.7	32.9	–	0.62
Experiment 2							
'Glenelg'	8.1[ab]	4.2[a]	43.3[a]	12.5[a]	31.9[a]	0.51[a]	0.72[a]
'M1589'	8.0[a]	4.0[a]	42.7[a]	23.8[b]	21.5[b]	0.51[a]	0.48[b]
F_1	7.7[b]	4.3[a]	44.0[a]	17.4[c]	26.6[c]	0.50[a]	0.61[c]
MP value	–	–	–	18.2	26.7	–	0.60

Within experiments means in the same column having a superscript in common are not significantly different at the 5% level

ments the major differences between 'Glenelg' and the mutant lines were the relative proportions of linoleic and linolenic acids, and hence also their ratio, LDP. The contents of linoleic and linolenic acids were inversely related.

In both crosses the F_1 means for linoleic and linolenic acids and LDP were intermediate between those of the respective parental lines, and significantly different from both. The F_1 means for each of these characters corresponded closely to the mid-parent value in both crosses, suggesting that co-dominant (additive) gene action was involved. Single gene hypotheses were tested by comparing the observed segregations in the backcross generations with those expected for the segregation of co-dominant alleles at a single locus. Although identical conclusions were obtained from the analysis of linoleic acid, linolenic acid and LDP, only the results for LDP are presented as the segregation patterns are clearer for this parameter. This is probably due to the fact that LDP is a more accurate measure of linoleic desaturation activity than is either linoleic or linolenic acid percentage, the absolute values of which are also affected by variation in the previous biosynthetic steps.

Based on the appearance of break points in the LDP frequency distributions (Figs. 1 and 2), plants were assigned to three phenotypic classes, viz. 0.38–0.52, 0.53–0.65 and 0.66–0.76 (Table 2). The inferred genotypes of these classes are also shown in Table 2; the symbols *a* and *b* refer to the mutant alleles present in 'M1589' and 'M1722' respectively, *A* and *B* being the

Table 2. Observed segregations for LDP in the backcross generations of the crosses 'M1589' × 'Glenelg' and 'Glenelg' × 'M1722' compared to those expected on the basis of a single additive gene

LDP class and inferred genotype constitution	Observed frequency[a]	
Experiment 1		
	F_1 × 'M1722'	F_1 × 'Glenelg'
0.38–0.52 (*bb*)	9 (7.5)	–
0.53–0.65 (*Bb*)	6 (7.5)	6 (5)
0.66–0.76 (*BB*)	–	4 (5)
Total	15 (15)	10 (10)
Chi-square (1 df)	0.60 ns	0.40 ns
Experiment 2		
	F_1 × 'M1589'	F_1 × 'Glenelg'
0.38–0.52 (*aa*)	14 (10)	–
0.53–0.65 (*Aa*)	6 (10)	11 (10)
0.66–0.76 (*AA*)	–	9 (10)
Total	20 (20)	20 (20)
Chi-square (1 df)	3.20 ns	0.20 ns

[a] Expected frequencies in brackets
ns = not significant at the 5% level

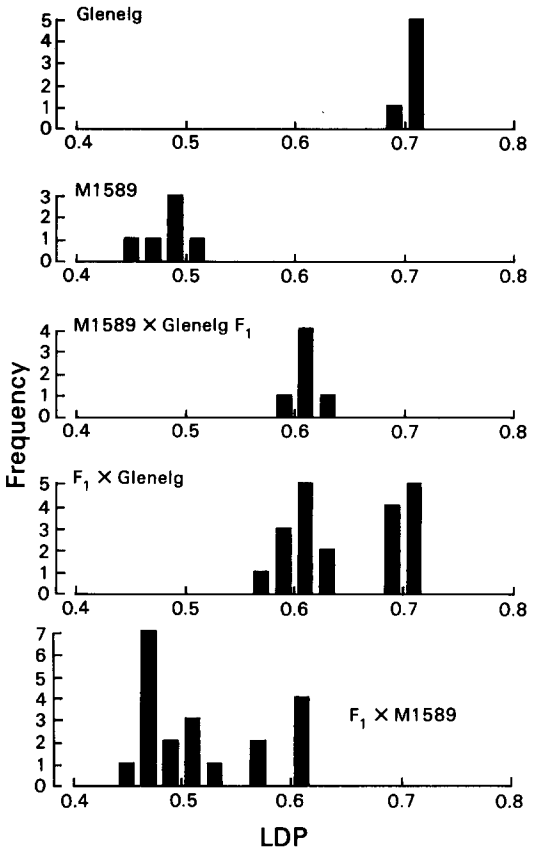

Fig. 1. Frequency distribution for LDP in the parental, F_1 and backcross generations of the cross 'M1589' × 'Glenelg'

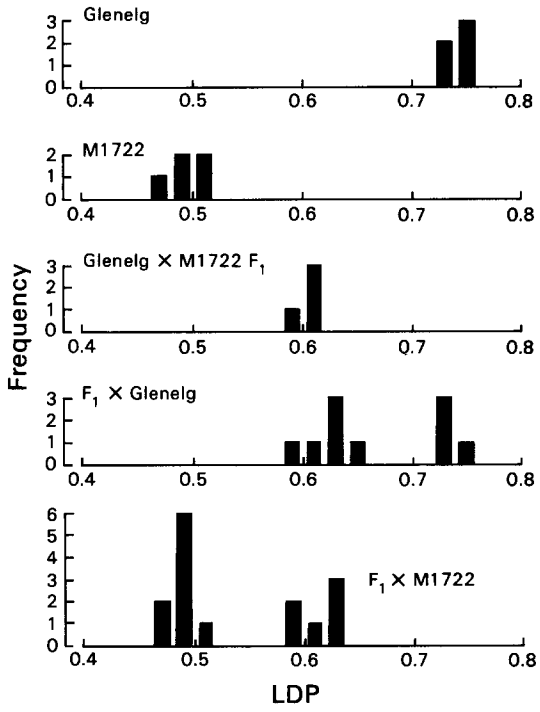

Fig. 2. Frequency distribution for LDP in the parental, F_1 and backcross generations of the cross 'Glenelg' × 'M1722'

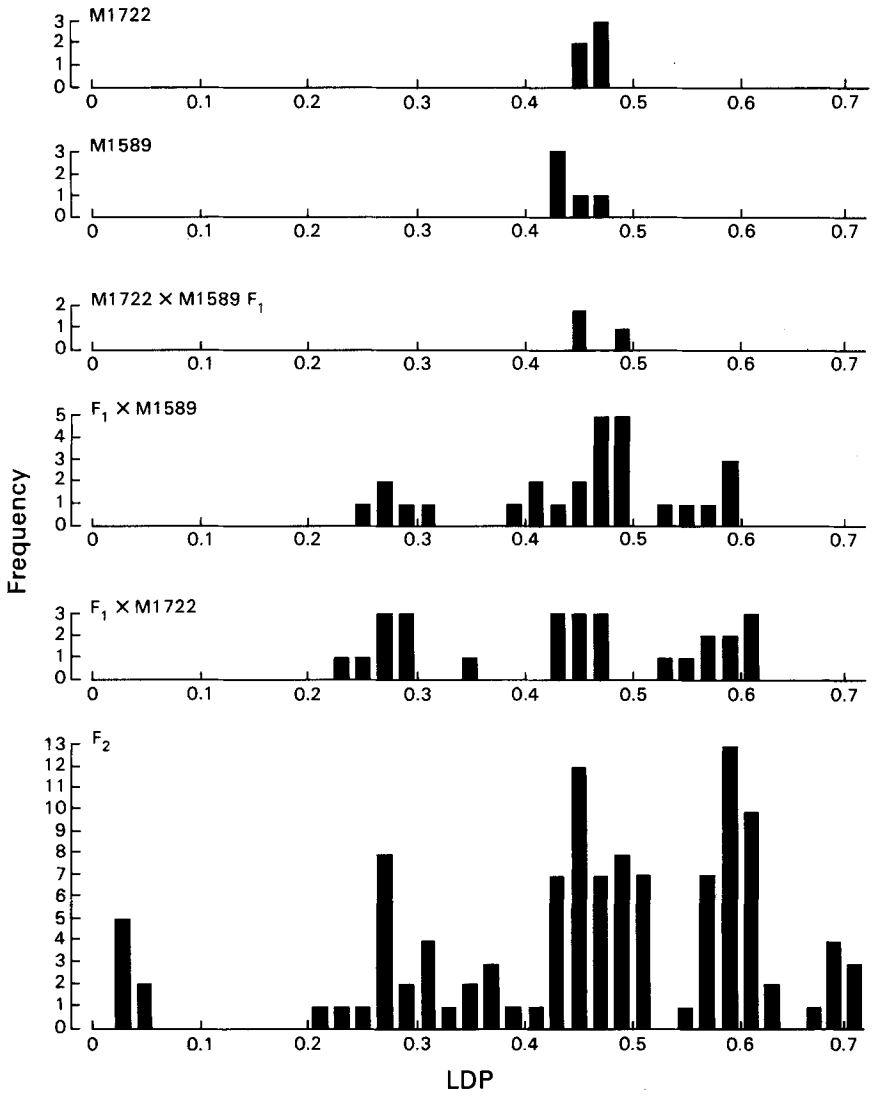

Fig. 3. Frequency distribution for LDP in the parental, F_1, F_2 and backcross generations of the cross 'M1722'× 'M1589'

Table 3. Mean fatty acid composition in the parental and F_1 generations of the cross 'M1722'×'M1589'

Genotype	Fatty acid composition (%)					ODP	LDP
	16:0	18:0	18:1	18:2	18:3		
'M1722'	8.4[a]	4.8[a]	35.4[a]	27.8[a]	23.3[a]	0.59[a]	0.46[a]
'M1589'	7.2[b]	5.0[a]	44.0[b]	24.5[b]	19.1[b]	0.50[b]	0.44[a]
F_1	7.4[b]	4.9[a]	37.6[a]	27.0[ab]	22.8[a]	0.57[a]	0.46[a]

Means in the same column having a superscript in common are not significantly different at the 5% level

normal alleles present at these loci in 'Glenelg'. Upper and lower case letters have been used only for clarity, not to indicate dominance. For both crosses the observed frequencies in the backcross generations do not differ significantly from the 1 : 1 ratio expected for the segregation of co-dominant alleles at a single locus.

Experiments 3 and 4

In order to determine whether the induced LDP mutants present in 'M1589' and 'M1722' are at the same or different loci, the cross 'M1722'×'M1589' was analysed. In Experiment 3, 'M1589' had significantly higher oleic and lower palmitic, linoleic and linolenic acid contents than M1722. Thus ODP was lower in 'M1589' than in 'M1722', but LDP was similar. The F_1 was similar to 'M1589' in palmitic acid content, and to 'M1722' in content of other fatty acids and ODP value. The F_1 generation was not significantly different from either parent for LDP (Table 3).

If 'M1722' and 'M1589' carry identical mutations in the same gene affecting LDP, then the F_1 genotype will be homozygous for this mutation and hence genetically identical to both parents, and the backcross and F_2 generations will not show genetic segregation. On the other hand, if the mutations are at different loci, the

'M1722'×'M1589' F_1 will be heterozygous at both loci and the backcross and F_2 generations will show segregation, the proportions of various genotypic classes being dependent on the degree of linkage between the two loci.

The frequency distributions for LDP in all generations of the cross 'M1722'×'M1589' are shown in Fig. 3. Segregation in the F_2 and backcross generations is present, with five classes apparent. The upper three classes correspond to those present in the 'M1589'× 'Glenelg' and 'Glenelg'×'M1722' crosses (see Figs. 1 and 2), whereas the two additional classes having lower LDP are novel. These classes indicate that the 'M1589' and 'M1722' mutations are in different genes, the lower LDP phenotypes arising by recombination of mutant alleles. Under this hypothesis the F_2 plants having an LDP of between 0.10 and 0.37 would be homozygous for the mutant at one locus and heterozygous at the other, whereas the plants in the lowest LDP class would be homozygous for the mutant alleles at both loci.

The observed LDP class frequencies in the F_2 and backcross generations are consistent with the segregation pattern expected for a hypothesis of two independently segregating genes acting additively to control the relative proportions of linoleic and linolenic acids (Table 4). Among the seven F_2 plants in the lowest LDP class, linoleic and linolenic acids averaged 50.6% and 1.6%, respectively. All seven low-linolenic F_2 plants were progeny tested by growing nine F_3 plants, all of which had fatty acid compositions similar to their F_2 parents, confirming the homozygosity of mutant alleles at both loci in this phenotypic class (Green 1986).

Table 4. Observed segregations for LDP in the backcross and F_2 generations of the cross 'M1722'×'M1589' compared to those expected on the basis of two independently segregating additive genes

LDP class	Inferred genotypic constitution	Observed frequency[a]
F_1×'M1722'		
0.10–0.37	1/4 *Aabb*	9 (6.75)
0.38–0.52	1/4 *AAbb*+1/4 *AaBb*	9 (13.5)
0.53–0.65	1/4 *AABb*	9 (6.75)
Chi-square (2 df) = 3.00 ns		27 (27)
F_1×'M1589'		
0.10–0.37	1/4 *aaBb*	5 (6.75)
0.38–0.52	1/4 *aaBB*+1/4 *AaBb*	16 (13.5)
0.53–0.65	1/4 *AaBB*	6 (6.75)
Chi-square (2 df) = 1.00 ns		27 (27)
'M1722'×M1589' F_2		
0.00–0.09	1/16 *aabb*	7 (7.1)
0.10–0.37	1/8 *Aabb*+1/8 *aaBb*	23 (28.5)
0.38–0.52	1/16 *AAbb*+1/16 *aaBB* +1/4 *AaBb*	43 (42.5)
0.53–0.65	1/8 *AABb*+1/8 *AaBB*	33 (28.5)
0.66–0.76	1/16 *AABB*	8 (7.1)
Chi-square (4 df) = 1.89 ns		114 (114)

[a] Expected frequencies in brackets
ns = not significant at the 5% level

Experiment 5

All possible combinations of homozygous and heterozygous genotypes at both mutant loci, produced by crossing 'Glenelg', 'M1589', 'M1722' and 'Zero' in a half diallel design, were analysed for fatty acid composition (Table 5). No substantial variation was present between genotypes for contents of palmitic, stearic or oleic acids, or for ODP value. However considerable variation was observed for linoleic and linolenic acids (Fig. 4), which were completely negatively correlated (r = −0.98), and hence also for LDP.

Genotypes were classed according to their number of mutant alleles at the two loci (Table 5). As expected, significant between-class differences were observed for linoleic acid, linolenic acid and LDP. LDP value declined progressively as the number of mutant alleles increased, but this reduction was not completely linear, with decrements of increasing magnitude as the number of mutant alleles increased. Similar trends were apparent for both linoleic acid and linolenic acid. Although this departure from complete additivity for LDP is only small, being approximately 15% of the average allelic substitution value at each locus, it suggests that the normal alleles may be partially dominant over the mutant alleles. The values for the heterozygotes were actually means of 30 of their F_2 offspring. Because in the presence of dominance the mean of a sample of F_2 seeds deviates from the mid-parent value by only half as much as does the true genetic value of

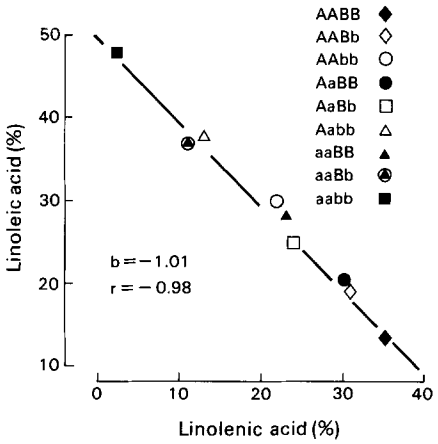

Fig. 4. Relationship between linoleic and linolenic acid contents in all possible genotypes at the 'M1589' and 'M1722' mutant loci

Table 5. Fatty acid composition of all genotypes at the two mutant LDP loci

Genotype and origin	Fatty acid composition (%)					ODP	LDP
	16:0	18:0	18:1	18:2	18:3		
No mutant alleles							
AABB 'Glenelg'	8.5	4.8	37.9	14.7	34.1	0.57	0.70
1 mutant allele							
AaBB 'Glenelg' × 'M1589'	8.1	4.7	37.3	20.7	29.2	0.57	0.59
AABb 'Glenelg' × 'M1722'	8.0	4.7	38.0	20.3	29.0	0.57	0.59
2 mutant alleles							
AaBb 'Glenelg' × 'Zero'	8.6	4.6	37.0	27.0	22.8	0.58	0.46
aaBB 'M1589'	7.6	5.4	37.8	27.6	21.6	0.56	0.44
AAbb 'M1722'	8.4	6.2	35.1	28.9	21.4	0.59	0.43
3 mutant alleles							
aaBb 'M1589' × 'Zero'	8.5	4.3	37.5	37.1	12.6	0.57	0.25
Aabb 'M1722' × 'Zero'	8.4	4.6	37.4	36.8	12.8	0.57	0.26
4 mutant alleles							
aabb 'Zero'	9.2	4.7	36.3	48.2	1.6	0.58	0.03

the heterozygous F_1 genotype, the true degree of dominance can be estimated at about 30% per locus in this experiment.

Within the classes having 1 or 3 mutant alleles, the contents of linoleic and linolenic acids, and the value of LDP, were similar, illustrating that allelic substitutions at either locus have equal effect. However, within the class having 2 mutant alleles, LDP was slightly, but significantly, higher in the heterozygous genotype (*AaBb*) than in the homozygous genotypes (*AAbb* and *aaBB*), again suggesting that the normal alleles are partially dominant over the mutant alleles.

Proposed gene symbols

For clarity during presentation of results the symbols *a* and *b* were used to represent the mutant alleles controlling LDP in 'M1589' and 'M1722' respectively. The use of upper and lower case symbols normally indicates complete dominance, and, because these genes have now been shown to exhibit basically additive (codominant) gene action, it is necessary to assign more appropriate symbols. It would also be desirable to choose symbols that are indicative of the functions of the genes; thus, because both linoleic and linolenic acids are altered equally by the alleles at these two loci, the gene symbols *Ln1* ('M1589') and *Ln2* ('M1722') are proposed. The *Ln* symbol has been previously assigned to genes controlling linoleic acid content in maize (de la Roche et al. 1971) and has not previously been used in *Linum usitatissimum* (Beard and Comstock 1965). Alleles in the mutant lines should be designated by the superscript 0, with those present in cv. 'Glenelg' being assigned the superscript 1. Any subsequently identified allelic variants would be classified by successive numerals. Under this system the homozygous genotypes in this study would be designated as follows:

'Glenelg' $Ln1^1Ln1^1\ Ln2^1Ln2^1$
'M1589' $Ln1^0Ln1^0\ Ln2^1Ln2^1$
'M1722' $Ln1^1Ln1^1\ Ln2^0Ln2^0$
'Zero' $Ln1^0Ln1^0\ Ln2^0Ln2^0$

Discussion

Genetic control of linoleic and linolenic acid content

The mutants analyzed in this study illustrate that in *L. usitatissimum* cv. 'Glenelg' the desaturation of linoleic acid to linolenic acid can be controlled by two independently-segregating major genes, *Ln1* and *Ln2*, each allele at either locus being responsible for an increase of approximately ten percentage points in linolenic acid content. Results from the diallel crosses indicated that the normal alleles $Ln1^1$ and $Ln2^1$ were partially dominant over the mutant alleles $Ln1^\circ$ and $Ln2^\circ$ respectively. However, codominance was apparent in the crosses 'Glenelg' × 'M1722' and 'M1589' × 'Glenelg'. In either case, heterozygotes were readily identifiable from homozygotes, meaning that for breeding purposes the alleles can be regarded as being codominant.

Such major gene effects have not previously been reported for fatty acid composition in flax or for linolenic acid content in any species. In contrast, genes of minor effect have generally been considered to be responsible for the limited variation observed in linolenic acid content in flax (Das and Rai 1974; Doucet and Filipescu 1981) and in other oilseed species such as rapeseed (Kondra and Thomas 1975) and soybean

(White et al. 1961). The relatively small differences in linolenic acid content that exist between 'Glenelg' and the majority of current flax varieties are presumably due to the cumulative effects of several minor genes that modify the expression of the two major genes identified in the present study. Some varieties of flax have much higher levels of linolenic acid than does cv. 'Glenelg'. For example cv. 'Avantgarde' has 64% linolenic acid, almost 20% higher than cv. 'Glenelg' (Green and Marshall 1981). A third major gene may be responsible for this large increment in linolenic acid content. Alternatively, 'Avantgarde' may carry different alleles at the *Ln1* and/or *Ln2* loci that result in greater linoleic acid desaturation activity than those present in 'Glenelg'. Further genetic studies are necessary to resolve this point.

The existence of two unlinked genes acting additively and equally to determine linolenic acid content in flax, is similar to the genetic system controlling erucic acid synthesis in *Brassica* species. In the diploid species *B. campestris* (genomic formula *AA*), a single gene controls the elongation of oleic acid (C18 : 1) to form eicosenoic (C20 : 1) and subsequently erucic (C22 : 1) acids (Dorrell and Downey 1964), whereas in the two alloploid species *B. napus* (*AACC*) and *B. juncea* (*AABB*), erucic acid is controlled by alleles at two unlinked loci (Harvey and Downey 1964; Kirk and Hurlstone 1983). It has therefore been postulated that each of the basic diploid *Brassica* genomes (*A, B, C*) carry a single gene, possibly homologous, controlling the elongation of oleic acid (Kirk and Hurlstone 1983). Since *L. usitatissimum* (2n = 30) is also considered to have a polyploid origin, it could be similarly suggested that the two major genes controlling linoleic desaturation represent duplication of a single gene present in the basic x = 9 *Linum* genome.

Although no variants for oleic desaturation have been reported, the possibility that major genes also control the previous desaturation of oleic acid to linoleic acid in *L. usitatissimum* is suggested by studies involving other oilseeds. In safflower, sunflower and maize, wide ranges in the proportions of oleic and linoleic acids have been demonstrated to be determined by major genes. Normally safflower has a high linoleic (75–80%) and low oleic acid content (10–15%), but types having low linoleic (12–30%) and high oleic (64–83%) have been identified. These differences in composition were demonstrated to be under the control of a single genetic locus with three alleles *Ol, ol*^{1} and *ol* (Knowles and Hill 1964). Similar variations in oleic and linoleic acids have been identified in sunflower, also normally a high-linoleic, low-oleic oil (Downey and Dorrell 1971). In maize, the 20% difference in oleic and linoleic acids between related lines R84 and IHO is controlled by a single gene, the *In* locus, high oleic acid content being dominant over low (de la Roche et al. 1971). Thus, as is the case for linoleic desaturation, the desaturation of oleic acid in flax is probably under simple genetic control, which might make possible the inactivation of this step by induced mutation breeding to achieve further large modifications in the relative proportions of oleic, linoleic and linolenic acids. Alternatively, minor modifications should be achievable by utilizing the variation for ODP already present in flax germplasm collections (Green and Marshall 1981).

Biosynthesis of linoleic and linolenic acid

Studies of triglyceride biosynthesis in developing flax cotyledons have concluded that oleic acid esterified to phosphatidylcholine (oleoyl-PC) is the substrate for sequential desaturation to form linoleoyl-PC and subsequently linolenoyl-PC (Slack et al. 1978; Stymne and Stobart 1985). The present results in which mutants having greatly reduced levels of linolenic acid had correspondingly elevated levels of linoleic acid, but little alteration to other fatty acids, supports this conclusion and offers strong evidence that separate substrate-specific enzymes are responsible for the individual desaturation reactions. Furthermore, the similar strong inverse relationship between these two fatty acids in the seed oils of wild *Linum* species (Green 1983) suggests that this pathway operates in the genus as a whole.

Two other pathways had previously been postulated to account for the synthesis of linolenic acid in plants. Kannangara and Stumpf (1972) concluded that linoleic and linolenic acids in spinach leaves were both produced directly from oleic acid by separate pathways, with linolenic being formed by the simultaneous removal of four hydrogens. Under this hypothesis, a large reduction in linolenic acid would be expected to result mainly in increased oleic acid content, with a partial flow-on into the linoleic acid pathway; such a pattern was not observed in the mutant genotypes of the current study. The other suggested pathway is that linolenic acid (C18 : 3) is produced by elongation of cis-3,6,9-dodecatrienoic acid (C12 : 3) (Kannangara et al. 1973). However, the expected precursor compounds, shorter chain trienoic acids, were not produced in response to reductions in linolenic acid in the current study and Oulaghan and Wills (1976) were also unable to detect any such compounds during flax seed development. Thus, neither of these two suggested pathways appears to play a significant role in the synthesis of linolenic acid in seed lipids in *L. usitatissimum*.

The origin of the 2% linolenic acid remaining in the recombinant mutant line is of interest. Thies (1970) noted that accumulation of linolenic acid occurs only in the seed of oil-bearing plants which possess green and photosynthetically-active chloroplasts during their development, such as those of the families *Brassicaceae* (rapeseed, mustard), *Fabaceae* (soybean) and *Linaceae* (flax). Thies also observed a positive correlation between the chlorophyll content and the amount of linolenic acid present in mutant rapeseed genotypes. Because linolenic acid is known to be the principal fatty acid of the chloroplast thylakoid membranes, he

considered that genotypes completely devoid of linolenic acid were unlikely to be obtained in these chloroplast-containing species. This conclusion is substantiated by the fact that although linolenic acid has been reduced in breeding lines of rapeseed (Röbbelen and Nitsch 1975), soybean (Wilcox et al. 1984). and now linseed, in no case has it been eliminated. The 1–2% level reported in the 'Zero' linseed genotype in the current study is the lowest yet produced in these species. It might therefore be postulated to represent the amount of linolenic acid associated with chloroplast structure or resulting from chloroplast activity, and hence be the biological limit to selection in such species. Lipid analysis of separated chloroplasts during seed development may help to resolve this point.

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