N.Z. Agronomy Society
Special Publication No. 5

Paper 47

GENETIC CONVERSION OF LINSEED OIL FROM INDUSTRIAL TO EDIBLE QUALITY

A.G. Green
Division of Plant Industry, CSIRO
Canberra, Australia

ABSTRACT

Linseed (flax) is an oilseed crop that is widely adapted to temperate climates but which is declining in importance because of the use of synthetic substitutes in traditional linseed oil markets. High levels of linolenic acid (45-65%) currently preclude the use of linseed as an edible oil because of flavour reversion problems.

Following EMS treatment of cv. Glenelg seeds, two mutants, M1589 and M1722, having reduced levels of linolenic acid (28-30%) were isolated. The mutations are in unlinked genes and exhibit additive gene action. By recombining the M1589 and M1722 mutations into a single genotype, linolenic acid content has been further reduced to around 1%, a level which results in greatly improved flavour stability.

The reduction in linolenic acid content is associated with an equivalent increase in that of the highly desirable linoleic acid to 50-70%, depending on temperature during seed maturation. Proportions of other fatty acids remain unaltered. The mutations thus appear to block the synthesis of linolenic acid by desaturation of linoleic acid.

The additive gene action, large phenotypic differences between genotypes, and absence of maternal effects, combine to simplify backcross breeding aimed at transferring edible oil characteristics to other linseed varieties.

KEYWORDS

Linolenic acid, linoleic acid, oxidative stability, induced mutation.

INTRODUCTION

Linseed oil, obtained from the seed of the flax plant (*Linum usitatissimum* L.), is unique among the major vegetable oils in that it contains a high level (45-65%) of α-linolenic acid (cis-9,12,15-octadecatrienoic). This polyunsaturated fatty acid is highly susceptible to oxidation and polymerisation, and imparts a drying quality to the oil which has made it important in the production of paints, varnishes, inks and linoleum. However, the market for linseed oil has declined dramatically in recent years as synthetic compounds have increasingly replaced linseed oil in the manufacture of these products.

In contrast, the market for edible vegetable oils is large and continually expanding. World production has almost doubled from 23 to 44 million tonnes between 1968 and 1981 (Boelhouwer, 1983). Linseed oil is not currently used as an edible oil because the autoxidation property of linolenic acid results in the formation of off flavours during storage. Experiments in which the linolenic acid content of soybean oil has been varied suggest that flavour stability increases as the linolenic level decreases below 3%, but for good flavour stability a linolenic acid content below 1% is considered necessary (Cowan *et al.*, 1970). In oils containing relatively low levels of linolenic acid, such as soybean and rapeseed, flavour reversion can be prevented by the process of hydrogenation, in which linolenic acid is converted to more stable, more saturated fatty acids. However, this procedure is not practical for reducing linolenic acid in linseed oil since it is costly, non-specific, and can lead to the production of nutritionally undesirable *trans*-isomers, as well as another flavour reversion factor, *iso*-linoleic acid.

A more satisfactory and permanent way to convert linseed oil to an edible oil is by the genetic removal of linolenic acid. A plant breeding programme with this objective commenced at CSIRO Division of Plant Industry in 1979. Natural variation for fatty acid composition assessed in a germplasm collection was considered to be insufficient to significantly reduce linolenic acid content by intraspecific hybridisation and selection (Green and Marshall, 1981). In contrast, wild *Linum* species varied widely in their fatty acid composition with several species having very low levels (<3%) of linolenic acid (Green, 1983). These species could not be exploited because of strong reproductive barriers preventing interspecific hybridisation (Green, unpub. data). Subsequent efforts therefore concentrated on mutation breeding with the aim of inactivating the desaturase enzyme(s) involved in the synthesis of linolenic acid in the seed oil.

METHODS AND RESULTS

Induction and selection of mutants

The availability of a rapid and sensitive chemical test

specific for the presence of linolenic acid in vegetable oils enabled the screening of large populations of seeds. This assay, known as the TBA test, relies on the formation of a red colour complex when TBA (thiobarbituric acid) reacts with the oxidation products of linolenic acid. Where linolenic acid is absent a yellow colour is observed, grading through orange to red as linolenic acid content increases (McGregor, 1974). Quantification is achieved by comparison to standards of known linolenic acid content.

Seeds of the current Australian linseed cultivar, Glenelg, were treated with various doses of either gamma radiation or ethylmethanesulphonate (EMS), using standard techniques (Green and Marshall, 1984). Ten M_2 seeds from each of 7072 field-grown M_1 plants were assayed non-destructively using the TBA test. Seeds appearing to have less than 40% linolenic acid were selected and progeny tested as M_2 plants. Two mutants were subsequently identified, M1589 and M1722, in which linolenic acid constituted 29% of the total fatty acids compared with 43% in seed oil from untreated Glenelg plants. The reduced level of linolenic acid was accompanied by an increase in the level of linoleic acid to 30%, compared with 18% in Glenelg, but there were no significant changes in the proportions of other fatty acids. Both mutants arose from 0.4% EMS treatment but from different M_1 plants.

Genetic analysis of M1589 and M1722

The inheritance patterns of the induced mutants were examined to determine whether they were allelic or in different genes that might be recombined to give even lower levels of linolenic acid. Analysis of the parental, F_1, and backcross generations of the crosses M1589 x Glenelg, and Glenelg x M1722 indicated that both M1589 and M1722 carried a single gene mutation for which the normal and mutant alleles were codominant (Green, 1985).

Analysis of 114 F_2 plants from the cross M1722 x M1589 revealed wide variation in linolenic acid content. Five distinct classes were apparent with means 1.6%, 15.0%, 24.0%, 30.5% and 34.5%. The frequencies of F_2 plants in these classes were 7, 23, 43, 33 and 8 respectively, which agrees with the genetic ratio of 1:4:6:4:1 (X^2_4 = 1.89 ns) expected for two unlinked loci exhibiting additive gene action. The two lower linolenic acid classes arise by recombination of the M1589 and M1722 mutants; the seven F_2 plants having less than 2% linolenic acid were confirmed as homozygotes for the mutant alleles at both loci by F_3 progeny tests. This double-mutant homozygous genotype has been referred to as Zero. The additional reduction in linolenic acid content was again accompanied by an equivalent increase in linoleic acid. This further demonstated that the M1589 and M1722 mutations specifically affect the linoleic desaturation step in fatty acid biosynthesis.

Using the M1589 and M1722 mutants it is now possible to specify three different linseed fatty acid compositions in pure-breeding (homozygous) material: the normal type having low linoleic and high linolenic, an opposite type having high linoleic and low linolenic, and an intermediate type having about equal proportions of both fatty acids (Table 1).

Table 1. Fatty acid composition of seed oil from linseed cv. Glenelg and induced low-linolenic acid mutants, grown in a glasshouse at 27/20 °C (day/night).

	Fatty acid composition (%)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Glenelg	8.5	4.8	37.9	14.7	34.1
M1589	7.6	5.4	37.8	27.6	21.6
M1722	8.4	6.2	35.1	28.9	21.4
Zero	9.2	4.7	36.3	48.2	1.6

Temperature sensitivity

The initial testing and genetic analysis of the mutants was conducted in a relatively high temperature glasshouse environment (27 °C day/20 °C night). Under these conditions, the composition of the seed oil of the Zero genotype (Table 1), approached that of high-linoleic sunflower, a premium-quality polyunsatured edible oil. The minimum industry requirement for the manufacture of polyunsaturated margarines is 62% linoleic acid. Therefore, a further increase in linoleic acid content would be necessary before the low-linolenic linseed oil could be used extensively in such products.

Because low temperatures during seed maturation result in a more polyunsaturated fatty acid composition in several oilseed species, including high-linolenic linseed, a Phytotron study was conducted to determine the effect of temperature on fatty acid composition of Glenelg and the homozygous mutant genotypes M1589, M1722, and Zero.

In all four genotypes, low temperatures resulted in decreased contents of palmitic, stearic and oleic acids, and increased contents of linoleic and linolenic acids. This was

Table 2. Effect of temperature during seed maturation on fatty acid composition of the low-linolenic linseed genotype Zero.

Day/night temperature (°C)	Fatty acid composition (%)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
27/22	8.5	6.3	32.1	50.9	1.9
24/19	8.1	6.0	28.0	55.9	1.8
21/16	7.4	4.4	29.0	57.3	1.8
18/13	6.4	3.3	25.1	62.7	2.3
15/10	6.2	3.2	17.3	70.2	3.1

demonstrated to be due to a greater level of oleic acid desaturation at low temperatures, the subsequent linoleic desaturation step being highly insensitive to temperature. In Zero, linolenic acid content was 3% or less at all temperatures, and linoleic acid content was more than 62% when day/night temperature was less than 21/16°C (Table 2). Thus, this genotype should produce a premium-quality polyunsaturated edible oil, similar to sunflower and maize oils, when grown as a winter crop in temperate environments.

Oxidative stability

A three litre sample of oil was refined and analysed by Vegetable Oils Pty Ltd. The crude oil was obtained by solvent extraction of Zero seed harvested from a field nursery plot grown in Canberra between September 1984 and January 1985. Processing of the oil consisted of degumming, alkali refining, bleaching, and steam deodorising. This combination produced a bland oil of light colour, with physical and chemical properties equivalent to those of typical sunflower oil (Table 3).

Oxidative stability was determined by an A.O.M. test, using the IUPAC Method 11D21, in comparison with high-linolenic linseed oil and soybean, rapeseed, and sunflower oils (no antioxidants added). The results indicated that the low-linolenic linseed oil is suitable for use as an edible oil, having similar stability to sunflower oil and soybean oil and being far more resistant to oxidation than 'normal' linseed oil (Fig. 1).

Future breeding objectives

The Zero genotype would appear to be suitable for direct use as a cultivar in those Australian environments in which Glenelg has been successfully cultivated. Agronomic testing is currently underway to confirm this. Conversion of cultivars adapted to other environments to low-linolenic acid content can be readily achieved by routine backcrossing procedures. The additive gene action, large phenotypic differences between genotypes, and absence of maternal effects (Green, 1985) combine to simplify the backcross breeding procedure.

Future breeding will concentrate on further improvements in oil quality of the low-linolenic genotype. To ensure that polyunsaturated oil quality standards are consistently met independently of temperature it would be desirable to develop a genotype having a temperature-stable linoleic acid pathway, similar to that of safflower. Alternatively, it might be possible to raise the level of linoleic acid genetically so that, although still temperature sensitive, high levels of linoleic acid (>62%) could be produced even under warm conditions. It is encouraging to note that several linseed and flax genotypes have significantly higher oleic desaturation activity than Zero. If this could be combined with the low-linolenic character, then the practical consequences of temperature sensitivity might be circumvented. A combination of these approaches might result in a linseed genotype capable of very high levels of linoleic acid synthesis under all temperature conditions.

CONCLUSION

The high-linoleic, low-linolenic linseed oil should be suitable for widespread use as an edible oil. At less than 2%, the level of linolenic acid is below that of commercial cultivars and currently available breeding lines of both soybean and rapeseed. Additionally, the large increase in linoleic acid content has resulted in an oil which is more polyunsaturated than either soybean or rapeseed, the composition and physical properties being similar to those of sunflower oil. The development of an edible-quality linseed oil greatly increases the market prospects for this crop and potentially provides expanded opportunity for diversification of cropping activities in many traditional cereal growing areas.

Table 3. Comparison of low-linolenic acid linseed oil with typical linseed and sunflower oils.

Property	Low-linolenic linseed	AOF Specifications[1] Linseed	AOF Specifications[1] Sunflower
Fatty acid composition			
Palmitic (%)	7	6	6
Stearic (%)	5	4	2
Oleic (%)	24	18	26
Linoleic (%)	63	19	66
Linolenic (%)	1	53	—
Free fatty acid (%)	0.18	max 0.25	max 0.25
Iodine value	133	175	125-138
Refractive index (25°C)	1.470	1.447-1.482	1.472-1.474
Relative density (25°C)	0.917	0.924-0.930	0.914-0.920
Saponification value	185	188-195	190-196
Unsaponifiable matter (%)	0.87	max 1.5	max 1.5
Peroxide value	nil	—	max 10

[1] AOF Oilseeds '82 (Australian Oilseed Federation, 1982).

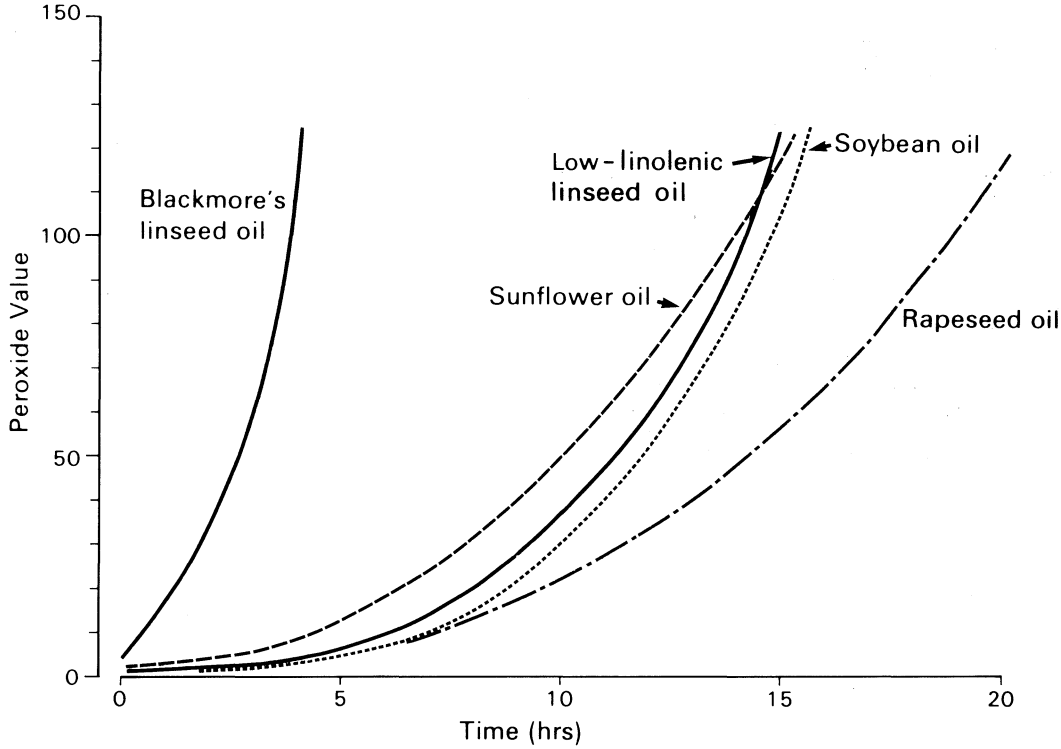

Figure 1. Oxidative stability of low-linolenic linseed oil compared to that of 'normal' linseed oil, sunflower oil, rapeseed oil and soybean oil.

REFERENCES

Boelhouwer, C. 1983. Trends in the chemistry and technology of lipids. *Journal American Oil Chemists' Society 60:* 409-414

Cowan, J.C., Evans, C.D., Moser, H.A., List, G.R., Koritala, S., Moulton, K.J., and Dutton, H.J. 1970. Flavour evaluation of copper-hydrogenated soybean oils. *Journal American Oil Chemists' Society 47:* 470-474.

Green, A.G. 1983. The occurrence of ricinoleic acid in *Linum* seed oils. *Journal American Oil Chemists' Society 61:* 939-940.

Green, A.G. 1985. Genetic modification of seed fatty acid composition in *Linum usitatissimum* L. PhD thesis (Australian National University).

Green, A.G., and Marshall, D.R. 1981. Variation for oil quantity and quality in linseed (*Linum usitatissimum*). *Australian Journal Agricultural Research 32:* 599-607.

Green, A.G. 1984. Isolation of induced mutants in linseed (*Linum usitatissimum*) having reduced linolenic acid content. *Euphytica 33:* 321-328.

SYMPOSIUM DISCUSSION

Dr K.R.W. Hammett, Division of Horticulture & Processing, DSIR

What was the method of mutagenesis? Was it irradiation?

Green

We tried gamma irradiation and EMS. The mutants came from an EMS treatment.

Dr D.S.C. Wright, Crop Research Division

How do the oil yields compare with the standard crop?

Green

We have not compared oil yields on a per hectare basis. The oil percentages are equivalent, but there is a factor we have not yet determined — the yield of the zero line in the field this year was only 70% of the yield of Glenell. That could be due to several reasons: there could be background mutations that we can select against in a back-crossing programme; it could be a pleotrophic effect of the mutations; or the mutations could have affected adjacent genes and had some effect on vigour. I do not expect it will be the

pleotrophic effect — these genes are very specific for the production of linolenic acid in seed lipids and they are not the ones that control, for instance, production of linolenic acid in the chloroplast lipids. Leaf lipids are of normal composition in the mutant lines.

Prof. D. von Wettsein, Carlsberg Laboratory

There seem to be two different pathways for producing linolenic acid. One where there is desaturation of linolenic acid; and another where the C12 acid is desaturated and then elongated. Have you any evidence that these two mutants are blocking the two different pathways or are they blocking the same pathway?

Green

The C12-3 to C18-3 pathway does not operate in linseed seed lipids. It has been suggested that it may operate at chloroplast lipid synthesis, but it has been looked at by other workers in linseed and does not operate. The complete correlation between linoleic and linolenic is strong evidence that only one pathway exists, and that is where the mutations are.