SHORT COMMUNICATION

Ethyl Methanesulphonate Induced Fatty Acid Mutations in Flax

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Seed of the flax cultivar McGregor were treated with the chemical mutagen ethyl methanesulphonate (EMS) with the objective of producing mutations that reduced the α-linolenic acid content of the seed. From the seed of 2430 M_1 plants initially examined by the thiobarbituric test, 53 were classified as having reduced linolenic acid levels. Utilizing half-seed analysis by gas chromatography, three lines taken through to the M_4 generation had reduced linolenic acid levels, but all three appeared to have different mutations. Mutant E67 has a palmitic acid level of 28.4%, three times the level found in McGregor. Mutant E1747 had a high linoleic content of 52.2%, and mutant E1929 had increased oleic and linoleic levels to twice that of McGregor.

Green and Marshall (1) reported the isolation from ethyl methanesulphonate (EMS) treated seed of Glenelg flax (*Linum usitatissimum* L.) of two mutant lines with reduced levels of α-linolenic acid. The linolenic acid content in seed of M_3 generation plants of these two lines was 10% less than seed of Glenelg. This was not low enough to classify these lines as an edible-oil flaxseed, which had been the objective of the EMS treatment. Subsequently, Green (2) described the recovery of F_2 plants from the cross of the two mutant lines, M1589 and M1722, whose seed contained less than 2% linolenic acid. This should allow the flax to become an edible oilseed. Green (3) showed that M1589 and M1722 each had a mutation in a different unlinked gene, and that these mutant genes showed additive gene action that produced stable 2% linolenic acid lines.

As these mutant genes were not available to us, and we wished to develop cultivars of edible-oil flax for Western Canada, we began our own flax mutation program at the Crop Development Centre. The results presented here describe the three most promising mutant low linolenic acid lines discovered to date.

MATERIALS AND METHODS

Twenty thousand seeds of the cultivar McGregor were treated with a 0.4% solution of EMS in a phosphate buffer pH 7.3, according to Green and Marshall (1). All of the seed was sown in the field at the Kernen Crop Research Farm, University of Saskatchewan, in May of 1987. In the autumn, 7,702 of the M_1 plants (i.e., plants arising from the EMS treated seed) were harvested.

Initial screening of linolenic acid content of the seed involved applying the thiobarbituric acid (TBA) test to 10 whole seeds from each M_1 plant (1). The work reported here was carried out on the first 2,430 plants. On the basis of the TBA test any plant suspected of having seed with reduced linolenic acid had 10-15 half-seeds analyzed for fatty acid content by gas chromatography (GC) (4). While the non-germ half of a seed was analyzed by GC, the germ half was germinated in a petri dish on an agar-water medium. Any half-seed that showed a reduced linolenic acid level had the germinated half transplanted to a pot containing a soil mixture. These M_2 plants (i.e., the second generation after EMS treatment) were then grown in a growth chamber at 20°C day, 15°C night with a 16-hr day.

Seed was harvested from each M_2 plant, and 10 half-seeds were analyzed on the GC and germinated as before. Again, seeds with reduced linolenic acid levels were grown in growth chambers under the same conditions. The work reported here extends to the M_4 generation on the three most promising lines discovered so far. The M_4 and parental fatty acid data were from whole seeds.

RESULTS AND DISCUSSION

Both the survival and fertility of the M_1 generation appeared better than in previous reports (1,5). Germination of the EMS treated seed was over 80%, and little complete sterility in M_1 plants was encountered.

The TBA test identified 53 lines with probable reduced linolenic acid levels. GC analysis on half-seeds from these 53 lines identified seeds with decreased linolenic acid levels, and germinating half-seeds were transferred to the growth chamber. Many of these lines are still being examined for novel fatty acid ratios, but three became of immediate interest.

Initially, we were interested only in the three major fatty acids of flaxseed—oleic, linoleic and linolenic. That was all that was recorded from seed of M_1 and M_2 plants. However, mutant E67, which had reduced levels of linolenic acid (Table 1) also had reduced levels of oleic and linoleic acid. A closer examination of seed from M_3 and M_4 plants revealed levels of palmitic acid three times greater than that found in McGregor.

Mutant E1747 was found to contain increased amounts of linoleic acid. This was the mutant line that most closely resembled those described by Green and Marshall (1). However, the stability of this line is in question as we found seed from M_4 plants with 2% linolenic acids. This variant is being further investigated.

The mutant line E1929, as with E1747, had reduced levels of linolenic acid. But rather than the increase being only in linoleic acid, there was a substantial increase in oleic acid as well.

The ODR and LDR further differentiate these mutants. The ODR of E67 and E1747 very closely resembled the ODR of McGregor, while the ODR of E1929 was reduced. None of the three mutants had an LDR that was similar to that of McGregor. The LDR of E67 was larger than that of McGregor, while those of E1747 and E1929 were lower.

It would thus appear that mutants E67 and E1929 are different from the two mutants described by Green

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TABLE 1

Fatty Acid Composition, Oleic Desaturation Ratio (ODR)[a] and Linoleic Desaturation Ration (LDR)[b] of Individual Plants of Three Mutant Lines of Flax and Their Parental Cultivar McGregor

		Fatty acid (wt% of total acids)						
Plant no.	Generation	Palmitic	Stearic	Oleic	Linoleic	Linolenic	ODR	LDR
E67	M_1			15.8	13.7	47.2	3.9	3.5
E67-2	M_2			14.8	4.7	41.8	3.2	8.9
-8				18.2	11.0	47.2	3.2	4.3
E67-2-5	M_3	26.2	2.3	11.3	5.7	46.4	4.6	8.4
-6		26.1	2.5	11.1	4.9	47.8	4.7	10.1
-7		23.4	3.1	18.4	6.7	36.9	2.6	6.4
E67-2-6-2	M_4	29.7	4.3	12.4	5.6	43.0	3.9	8.2
-4		27.0	3.3	14.6	7.0	42.9	3.7	6.2
E1747	M_1			17.4	19.6	48.3	3.9	2.5
E1747-5	M_2			17.9	16.4	49.3	3.7	3.0
-9				15.1	35.5	33.7	4.6	1.0
E1747-9-1	M_3	8.8	3.7	19.9	39.9	25.9	3.5	0.8
-5		9.6	3.7	24.7	44.5	15.8	2.6	0.4
-7		10.7	3.8	20.1	49.1	15.5	3.4	0.4
E1747-9-1-2	M_4	7.0	2.6	19.3	45.7	24.6	3.8	0.6
-3		7.2	3.3	16.1	54.8	18.6	4.8	0.4
-4		7.3	3.2	13.7	47.0	28.6	5.7	0.7
E1747-9-7-1		6.0	2.6	19.1	44.3	27.4	3.9	0.6
-3		6.3	2.6	17.4	58.8	14.5	4.4	0.3
-8		6.8	3.0	15.8	55.9	18.4	4.9	0.4
-9		6.8	3.2	14.7	58.9	16.4	5.4	0.3
E1929	M_1			24.4	16.9	44.4	2.5	2.6
E1929-3	M_2			25.9	12.1	46.9	2.3	3.9
-4				37.5	22.4	25.5	1.3	1.1
-5				34.9	10.8	39.6	1.4	3.7
E1929-4-1	M_3	10.6	3.2	39.9	23.5	21.6	1.3	0.9
-3		9.1	3.6	46.7	20.2	18.8	0.9	1.0
E1929-4-3-4	M_4	7.1	2.4	38.7	29.0	22.4	1.4	0.8
-6		7.3	2.3	34.7	30.9	24.6	1.7	0.8
-7		7.3	3.0	38.1	27.4	23.9	1.5	0.9
-8		6.8	3.1	27.2	33.1	29.7	2.6	0.9
McGregor-1	Parental	6.9	3.7	18.0	15.6	54.4	4.0	3.5
-2		6.8	3.9	18.4	16.3	53.5	3.9	3.3
-3		6.7	3.4	19.2	15.2	54.1	3.7	3.6
-4		6.8	3.9	16.1	15.9	56.1	4.5	3.6

[a] $\text{ODR} = \frac{\% \text{ linoleic} + \% \text{ linolenic}}{\% \text{ oleic}}$

[b] $\text{LDR} = \frac{\% \text{ linolenic}}{\% \text{ linoleic}}$

and Marshall (1). They give hope that the fatty acid ratio of flax may be further modified to meet specific requirements of the edible-oil market. Further work to characterize these mutants is progressing.

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