Opportunities and Problems in Modification of Levels of Rapeseed C_{18} Unsaturated Fatty Acids[1]

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ABSTRACT

Selections for different levels of C_{18} fatty acids in rapeseed to date have had only limited success, due in part to the low frequency of occurrence of desired genotypes with increased linoleic and decreased linolenic acid. In the progeny of mutation experiments with seeds of the variety Oro (linolenic acid content 8-10%) two stable mutants were selected, one with 5% and the other with 20% linolenic acid in the seed oil. The level of linoleic acid in the two mutants is the same as Oro (16-20%), but the levels of oleic and linolenic acids are inversely altered. In this paper several problems associated with selecting for linoleic and linolenic acids, which became apparent during the mutation studies, are discussed. Many selections made from the mutated material were unstable, reverting to the original Oro fatty acid composition after two or three self-pollinated generations. This fact plus environmental and maternal effects made selection difficult. However, with the use of rapid and simple analytical methods and space-saving growing techniques, these difficulties were overcome.

INTRODUCTION

Plant breeding work with rapeseed, aimed at improving seed quality, is at present directed towards improving both the oil through alteration of the fatty acid composition and the meal remaining after oil extraction (1,2). Two goals–lowering the levels of the objectionable long chain fatty acids eicosenoic and erucic, and reducing the content of undesirable glucosinolates–have been successfully achieved by plant breeding. In the *Brassica napus* species this success is due to selection from the variety Liho of the desirable genotype with only traces of eicosenoic and erucic acids (3), and the discovery that the variety Bronowski contains a low content of glucosinolates in the seed (4,5). From these materials high-yielding agronomically desirable varieties are under development. In *Brassica campestris* similar progress is being made as a result of the presence of genotypes low in eicosenoic and erucic acids (6) and the acquisition of low glucosinolate genotypes through interspecific crossing between *Brassica napus* and *Brassica campestris* (Pawlowski, unpublished).

For the development of varieties with the desired characteristics of low linolenic acid (<1%) and high linoleic acid (~50%) in the seed oil (7), suitable genotypes are not yet available. Thus identification of such genotypes within the population has a high priority in breeding programs. To date emphasis has been placed on screening large numbers of plants within the many varieties and species of the Brassica genus (8). This approach has resulted in only limited genetic advance due to several factors such as the lack of a fast, accurate test for levels of linoleic and linolenic acid, the relatively low heritability of changed proportions of these acids because they are due to such factors as environmental conditions during seed development, and the relatively narrow ranges in linoleic and linolenic acid levels found in the populations studied.

Recent studies indicate that more rapid advances may be possible through the use of chemical mutagens to increase the range in genotypes available (9) and by applying the recently developed rapid, accurate analytical method, which provides the ratio of these two important fatty acids as an estimate of their levels (10). The use of the half-seed technique reduces the number of analyses needed for selection, and space-saving growing techniques allow for more efficient utilization of limited greenhouse space.

CHOICE OF MATERIAL

The synthesis of fatty acids in a developing rapeseed occurs by two different pathways (Fig. 1). Eicosenoic and erucic acids are synthesized by chain elongation from oleic acid, while linoleic and linolenic acids are synthesized by desaturation (11). Because of this common origin the levels of polyunsaturated acids in rapeseed are modified by the genetic capacity for eicosenoic and erucic acid synthesis. Therefore selection for different levels of C_{18} fatty acids may be more efficient in material, such as the variety Oro, that does not contain appreciable levels of eicosenoic or erucic acids in the seed oil because fatty acid synthesis is largely directed towards production of the polyunsaturated fatty acids, the amounts produced are increased and the difference between the amounts of linoleic and linolenic acids enlarged. In addition, because of the current objection to the presence of long chain fatty acids such as eicosenoic and erucic in edible oils, it is desirable to combine low linolenic and high linoleic with low contents of the longer chain fatty acids.

SELECTION METHODS

The frequency of occurrence of genotypes with decreased linolenic or increased linoleic is low. Even with the use of X-rays and the chemical mutagen ethylmethylsulfonate, it is no higher than 1 in 5000 (9). Therefore rapid and simple analytical methods are essential for successful selection of genotypes with altered polyunsaturated fatty acid composition in rapeseed. Current gas chromatography methods, by which the fatty acid composition of the oil is determined, are too time consuming for this purpose (12). Alternatively, a modification of a photometric method for the measurement of polyunsaturated fatty acids after alkaline isomerization, which was commonly used before gas chromatographic methods, is a simple and rapid method suitable for the investigation of large numbers of samples. Refinement of this method by the use of ethylene-glycol-dimethyl ether instead of glycerine or glycerol has not only

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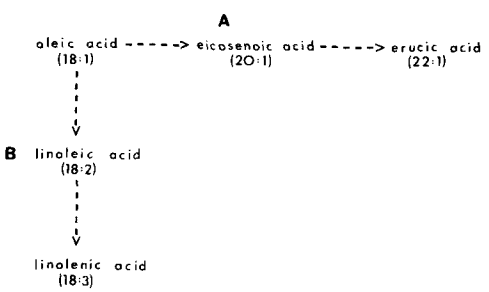

FIG. 1. Pathways of fatty acid synthesis from oleic acid in rapeseed. A = elongation, B = desaturation.

TABLE I

Content of C_{18} Fatty Acids in Seeds of Four Generations of the Mutants M57 and M364 Grown under Different Environmental Conditions (9)

Mutant	Generation[a]	Analysis no.	Fatty acids in % of total 18:1	18:2	18:3	% 18:3 / % 18:2
M57	Wi-M4	293	73.1	16.1	4.1	0.25
	Su-M5	130	65.9	19.6	6.9	0.35
	Wi-M6	126	72.8	15.3	3.4	0.22
	Su-M7	45	67.4	16.9	5.4	0.32
M364	Wi-M4	158	56.1	20.4	14.8	0.73
	Su-M5	92	51.0	21.7	19.1	0.88
	Wi-M6	91	58.9	21.3	10.0	0.47
	Su-M7	48	49.5	22.8	18.2	0.80

[a]Wi = winter, Su = summer; greenhouse conditions.

shortened the time required for the isomerization reaction, but also allowed the use of lower temperatures (13) and eliminated the need to carry the reaction out under inert atmospheric conditions (10). Used in conjunction with a recently developed micromethod for single rapeseed cotyledons, 300 analyses per day may be performed by one person (10).

With the photometric method, only the ratio between the linoleic and linolenic acids is obtained. This is not disadvantageous for selection because either lower linolenic, higher linoleic or both will result in lower ratios and these are all desirable changes. In addition, by using the ratio of linolenic acid to linoleic acid, rather than the per cent fatty acid composition of the oil usually calculated from gas chromatographic data, desired fatty acid compositions are more easily identified because the effects of environment on the normal biosynthetic process, which tend to alter the levels of linoleic and linolenic acids in concert, become less apparent (Tables I and II).

In addition to the photometric method, the technique of analyzing one cotyledon and growing a plant from the remaining embryo (14) may be applied to selection for linoleic and linolenic acids since, as was observed for eicosenoic and erucic acids (15), the levels of polyunsaturated fatty acids in rapeseed are largely determined by the genotype of the developing embryo (16); that is, the fatty acid composition of the analyzed seed characterizes the fatty acid composition of the seed of the following self-pollinated generation. Application of this half-seed technique was found to be very valuable for selection of different levels of linoleic and linolenic acids after mutagenic treatment, since it allowed selections to be made on the segregating M_2 half-seeds from M_1 plants. In this way, 98% of the analyzed seeds could be discarded (10) and greenhouse space required to grow further generations conserved by growing only those seeds with the desired fatty acid compositions.

It was also found that greenhouse space may be used more efficiently by growing these plants in small 6-7 cm diameter pots. Only the first 6-10 flowers per plant were self pollinated and all remaining flowers and branches were removed.

INSTABILITY OF SELECTED MUTANTS

A major difficulty encountered in selecting for modified polyunsaturated fatty acid compositions in rapeseed was reversion of the selections to the fatty acid composition of the original material. Many selections were made for different levels of linoleic and linolenic acid by analyzing M_2 half-seeds from M_1 plants grown from seeds treated with mutagens. Six examples of these are shown in Figure 2. Calculation of the per cent fatty acid composition from gas chromatographic data indicated that some selections had increased oleic acid levels (70%) and the same level of linoleic and linolenic (10%). Others had a decreased level of oleic acid (50%) and an increased level of linoleic acid (30%) accompanied by a normal level of linolenic acid (10%). Seeds of one selection were very high in linoleic acid (40%), had the same level of oleic acid (40%) and a normal level of linolenic acid (10%). However only a few of these selections continued to transmit their modified fatty acid composition through further generations. Many reverted to the original Oro type of fatty acid composition in the next generation. Some took an additional generation (17).

The cause of this reversion is unknown. It added to the selection work the requirement of repeated cultivation of selected plants through several self-pollinated generations in order to determine the stability of the fatty acid composition, and greatly increased the number of plants to be grown and analyzed, thus increasing the need for space-saving growing conditions and a rapid and simple fatty acid analytical technique.

ENVIRONMENTAL EFFECTS ON FATTY ACID COMPOSITION

Despite the problem of reversion two mutants, one with a lower linolenic acid content (M57) and the other with a

TABLE II

Content of C_{18} Fatty Acids in Seeds of Four Generations of the Mutants M57 and M364 Grown under the Same Environmental Conditions (9)

Mutant	Generation	Analysis no.	Fatty acids in % of total 18:1	18:2	18:3	% 18:3 / % 18:2
M57	M4	46	69.6	17.9	5.5	0.31
	M5	41	66.1	19.7	5.1	0.26
	M6	45	67.0	17.0	5.4	0.32
	M7	45	67.4	16.9	5.4	0.32
M364	M4	48	48.4	22.4	19.4	0.87
	M5	48	48.8	22.7	18.5	0.81
	M6	46	48.5	22.3	20.1	0.90
	M7	48	49.5	22.8	18.2	0.80

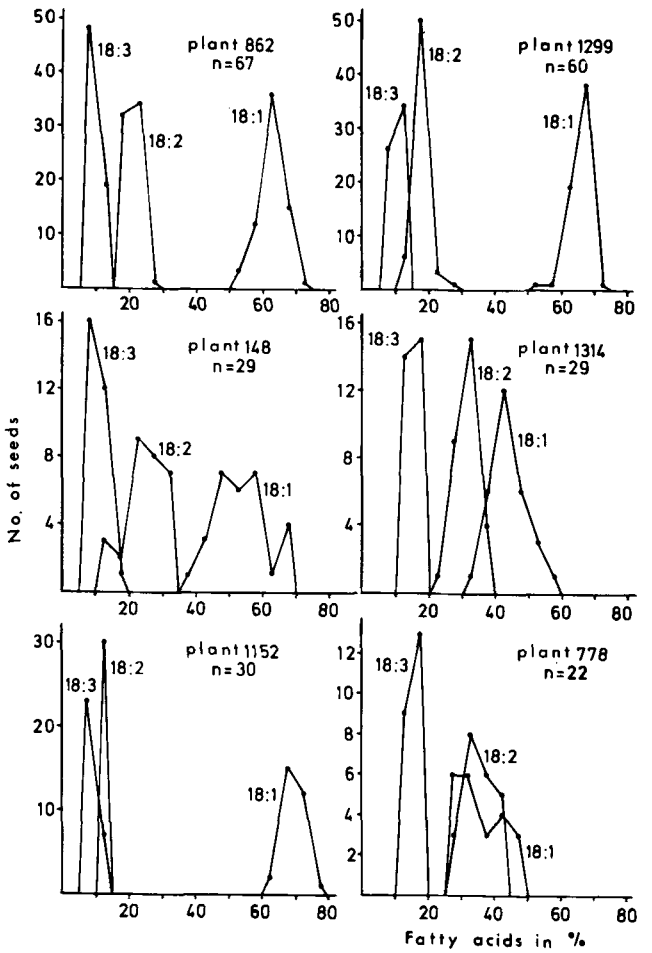

FIG. 2. Content of C_{18} fatty acids in % of total fatty acids from M_2 half-seeds from six different M_1 plants (9). *n* = number of half-seeds investigated.

higher linolenic acid content (M364), were selected as half-seeds from the segregating M_2 seed generation after mutagenic treatment of the variety Oro (9). To be certain of the stability of the modified fatty acid composition, a repeated cultivation of these mutants was performed. Table I shows the variation in the fatty acid composition of successive generations grown under summer and winter greenhouse conditions. The linolenic acid level was found to vary considerably from one generation to the next. The magnitude of the variation in M364 exceeds that in M57, but both fluctuated in concert and the level of linolenic acid in M364 always exceeded M57. In both mutants the level of linoleic acid was more or less stable.

When plants were grown from the seed of successive generations at the same time under the same greenhouse conditions (Table II), it became apparent that the fluctuations in the levels of linolenic acid were due to different summer and winter greenhouse conditions. It was also apparent that selection for genotypes with modified linolenic acid levels would be most effective under environmental conditions similar to the summer greenhouse conditions, which favor synthesis of linolenic acid. Summer conditions yielded larger differences in the per cent linolenic level between the two mutants and, since linoleic acid levels fluctuated little with the summer and winter greenhouse conditions, differences in the ratio of linolenic to linoleic between the mutants were larger when plants were grown under summer conditions.

More detailed studies on the environmental factors such as temperature and day length, which cause variation in the levels of polyunsaturated fatty acids, are needed before optimal environmental conditions for selection can be established.

TABLE III

Content of C_{18} Fatty Acids in Different Generations of Crosses between M57 and M364 (9)

	Generation	Fatty acids in % of total 18:1	18:2	18:3
P	M57 - M4	69.6	17.9	5.5
	M364 - M4	48.4	22.4	19.4
F_1	M57 X M364	66.8	16.1	7.9
	M364 X M57	61.3	18.0	9.7
	Average	64.0	17.0	8.8
F_2	M57 X M364	63.7	18.6	8.0
	M364 X M57	65.0	18.3	7.6
	Average	64.3	18.5	7.8
F_3	M57 X M364	65.5	17.7	6.9
	M364 X M57	65.5	17.3	6.9
	Average	65.5	17.5	6.9

Constant high temperature night and day has been observed to decrease levels of unsaturation in the seed oil of rapeseed (18), whereas high day temperature and low night temperature has been shown to increase unsaturation (19). The latter may explain the higher degree of unsaturation observed in the seed oil of plants grown under summer greenhouse conditions.

MATERNAL EFFECTS ON FATTY ACID COMPOSITION

Crossing experiments were carried out with the two mutants M57 and M364. The fatty acid compositions of the parents and of crossed generations are shown in Table III. The results indicated that the linoleic and linolenic acid levels are determined to a large extent by the genotype of the developing embryo. But reciprocal differences were observed for linolenic acid indicative of maternal effects. F_2 and F_3 seeds showed no maternal effects, but from F_1 through F_2 to the F_3 generation a continuous reduction of the linolenic acid level was observed. This may be due to better fertility of M57 over M364. Although differences due to maternal effects were significant at the 1% level, these were much smaller than differences in the linolenic acid levels caused by environmental effects and consequently were less of a problem in selecting for modified linolenic acid levels.

Similarly inheritance patterns have been observed for the linoleic acid content in rapeseed, which is to a large extent under embryonic control but is also influenced by the maternal plant (20).

The mutation experiments have indicated that it is possible to select for genetic variation in the levels of linoleic and linolenic acids in the self-pollinating *Brassica napus* species, and it is hoped that further work will result in obtaining genotypes with fatty acid compositions closer to the breeding goal of 50% linoleic and less than 1% linolenic. However, since the frequency of desired genotypes, even with the use of mutagens, is low and because of the influence of environmental effects plus the need to grow selections for several self-pollinated generations to ensure stability of the fatty acid composition, a large number of seeds will have to be analyzed. This may best be achieved by using the linolenic to linoleic acid ratio as determined by the photometric method since it is a simple and rapid method. The number of plants to be investigated and therefore the number of fatty acid analyses to be done may be reduced by employing the half-seed technique and the efficiency of the screening process improved by employing space-saving growing techniques.

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