CO1970RESEA10

SELECTION FOR FATTY ACIDS IN RAPESEED

By G. Röbbelen and G. Rakow
Institute of Plant Breeding,
University of Gottingen,
Fed. Rep. Germany.

Because of crop rotation problems the acreage of rapeseed has increased more than fivefold in Germany since 1955. It doubled in the last four years and the curve continues to climb. However, almost none of the crop is used for margarine production, and only cheap cooking and salad oils contain certain amounts of rapeseed oil. The main reason for this second-class rating of rapeseed oil is its insufficient nutritional quality. The fatty acid composition of rapeseed oil derived from the present varieties differs widely from the well-founded demands of the oil processing industry in Germany (Table I).

TABLE I

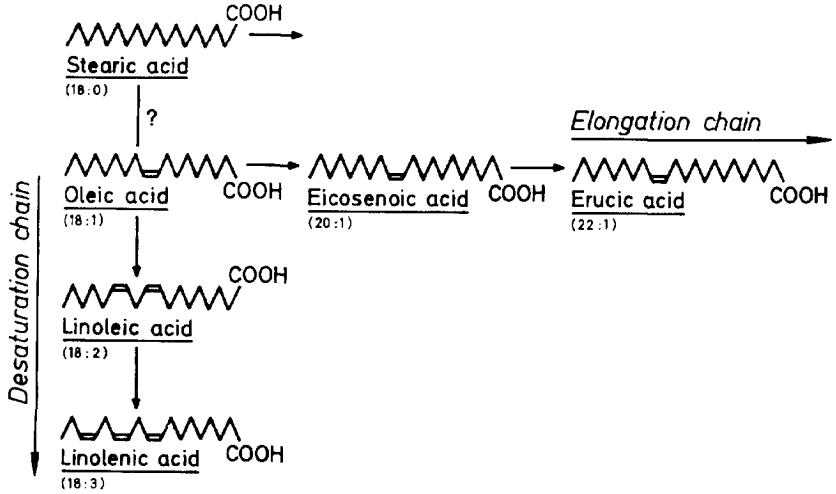
FATTY ACID COMPOSITION IN RAPESEED OIL

Fatty Acid		Content in Present Varieties	Desirable Content
Erucic	22:1	45%	0%
Linoleic	18:2	15%	>25%
Linolenic	18:3	11%	0%
Saturated	..:0	5-15%	5-15%
Oleic	18:1	Rest	Rest

The biosynthetic pathway towards the predominating types of fatty-acids, present in rapeseed oil (Figure I), is branched into two chains, the fatty acid elongation chain and the desaturation chain, both starting with oleic acid as a common precursor. An ideal edible vegetable oil is expected to be free of erucic and linolenic acid, but to contain as much linoleic acid as possible. Therefore, the plant breeder was asked for genetic blocks within both chains. The undeniable merit of STEFANSSON, HOUGEN and DOWNEY (1961) is the detection of such a blockade between the oleic and eicosenoic acid in the elongation chain. But a definite change in the desaturation chain, especially one interrupting the step from the linoleic to the linolenic acid, is so far not known in any rapeseed variety.

FIGURE I

BIOSYNTHETIC PATHWAY OF THE MAIN FATTY ACIDS IN RAPESEED



SELECTION IN THE ELONGATION CHAIN OF FATTY ACID BIOSYNTHESIS

The first "zero erucic" rapeseed genotype was found in the German variety "Liho". This spring type variety could more easily be fitted to the Canadian than to the European requirements of cultivation, as more than 90% of the German rapeseed is produced as a winter crop. Moreover, the original "Liho" was bred for use as a second crop for green manure and not for seed harvest. Therefore, zero erucic types, generously supplied by Dr. Downey to several breeders and institutions in Europe, were subjected to extensive back-crossing procedures with high-yielding native winter-rape varieties as recurrent parent. In this context we hardly need to emphasize the value of the "half-seed" technique, first described by Downey and Harvey (1963), for the early selection of erucic acid in segregating progenies; nor need we go into the technical details of the analytical screening methods by gas liquid chromatography, or paper chromatography applicable to large numbers of samples, which are indispensable for any promising breeding program. Recent improvements, which evolved during our work in Gottingen, will be summarized elsewhere (Thies, 1970)[b]. But a few factors, which also determine the efficiency of the backcrosses, may deserve some further attention.

Offsprings from "zero erucic" types, i.e. seeds with erucic acid less than 1% of the total fatty acids, always breed true. In all our crosses, ever done with conventional German winter rape varieties, the F_2 seeds segregated only one such zero erucic type among 46 individuals. This value stands for a total population of 21,600 seeds. Whatever the content of the non-zero erucic types is — the ratio indicates that more than one genetic locus is involved. Stefansson and Hougen (1964) postulated two independent loci,which display no dominance and act in an additive manner. Proof for this hypothesis was also given by Downey (1963), who found 17 (or 19) zero erucic genotypes among 404 F_2 individuals. In our F_2's, however, the output of zero erucic types is significantly less than the theoretical 1 among 16, pointing towards additional genetic determinants. At any rate we do not see, how Krzymanski and Downey (1969) justify the assumption of an allelic series for most of the recovered differences in erucic acid content. For in that case, the zero erucic allele should always segregate in a 3:1 ratio.

Figures II and III demonstrate a second problem of our backcrossing program, which is the recovery of a sufficient winter hardiness. In the F_1 the summer type of the "zero erucic Liho" is entirely dominant over the winter habit of the common German varieties, and more than 95% of the F_2 generation flower without cold treatment. This low ratio of the desired type drastically adds to the expenses of the screening efforts for erucic acid. In some experiments we have therefore tried first to select for winter type habit by sowing the F_2 seeds in late spring; the non-flowering types were transferred to a greenhouse in the fall and cold treated. But it turned out that this selection for winter type had additionally reduced the chance to recover zero erucic types (Figure II). Because of the polygenic nature of the winter type characteristic this result is easily explainable — even without assuming linkage. The same data were recovered by measuring the shoot elongation of 9 week old F_2 plants, which indicated the most early flowering to be in the zero erucic class (Figure III).

The last comment in connection with such backcrossing programs may stress the point, that the best oil quality will not satisfy in the end, if the final variety does not produce this oil in a sufficiently high amount. It has long been known, that the oil content of seeds is closely correlated with its specific gravity. Figure IV gives some preliminary data on a selection experiment with seeds of the Canadian zero erucic variety "Oro". In each of 3 consecutive generations the best and the lowest 10% of the seeds were separated in, respectively 15 and 30% sucrose solutions. The distinct selection progress, which we gained by this simple technique even in the positive direction, led us to use it as an obligatory routine procedure in many of our breeding schedules for rapeseed quality.

FIGURE II

CONTENT OF ERUCIC ACID IN F_2 PROGENIES OF THE CROSS "ZERO ERUCIC LIHO" X COMMON WINTER-TYPE RAPESEED, SELECTED FOR NON-FLOWERING(b) AFTER SPRING SOWING

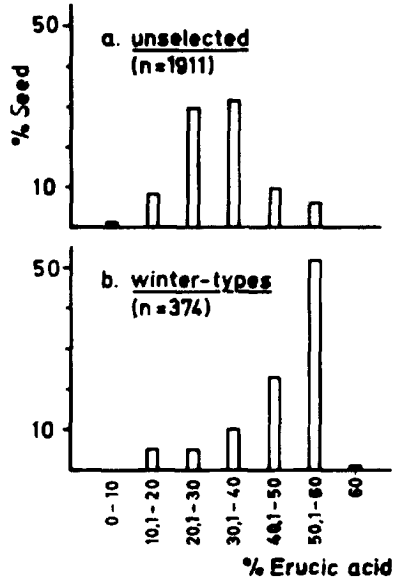


TABLE III

CORRELATION OF ERUCIC ACID CONTENT IN THE SEED WITH THE SHOOT LENGTH OF 9 WEEK OLD F_2 PLANTS ("ZERO ERUCIC" X COMMON WINTER-RAPE)

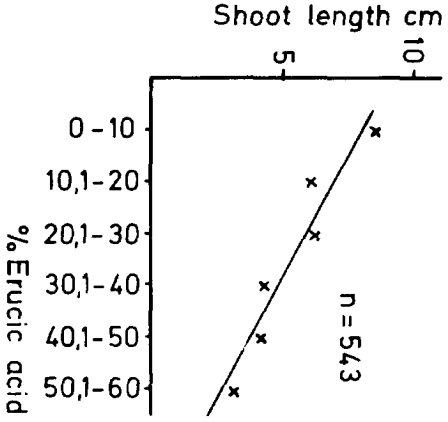
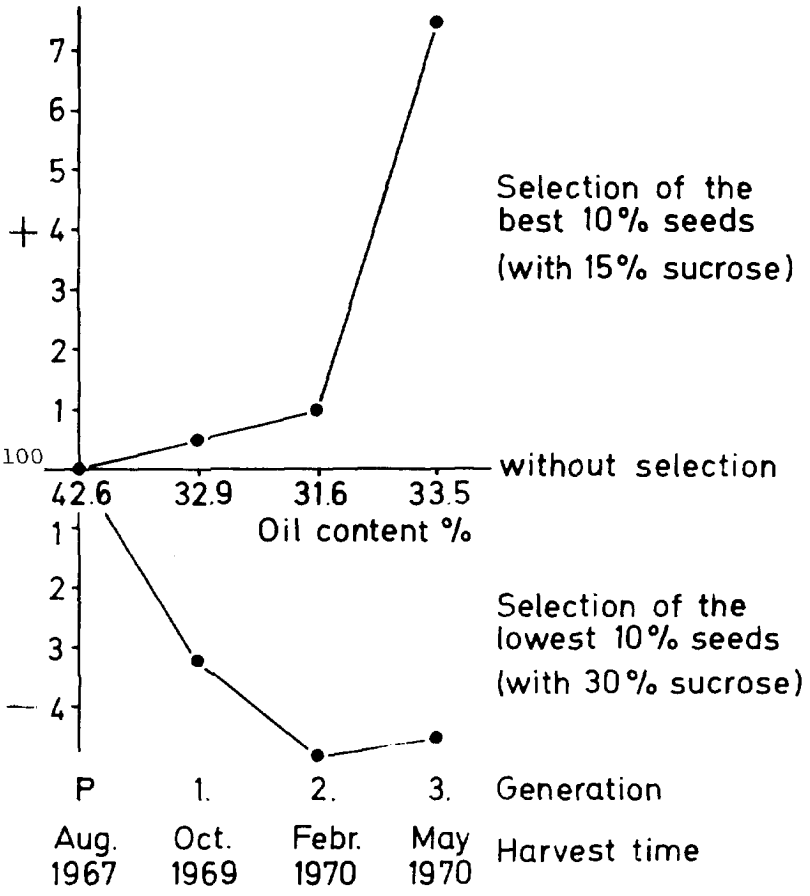


FIGURE IV

SELECTION FOR OIL CONTENT BY SUCROSE SOLUTIONS IN RAPESEED, VARIETY "ORO" (ATAKISI, UNPUBL.)



Although 3 backcrosses have been carried out up to now with the "zero erucic Liho" on several high yielding German winter rape varieties, the new lines, lacking erucic acid, are still inferior to the average of the present day German varieties. Table II gives some preliminary data from a small scale experiment in 1970, including our best lines with 1 m^2 plots without replication. The yield deficiency of the new winter types of zero erucic rapeseed against the standard variety "Lembkes" appears to be rather large. But the favourable conditions of the last winter season allowed even the summer variety "Oro" fully to survive. Thus severe winters may soon change this relatively optimistic outlook.

TABLE II

YIELD OF ZERO ERUCIC GENOTYPES IN COMPARISON TO THE COMMON STANDARD VARIETY "LEMBKES" WINTER RAPE . SOWING DATE SEPT. 2, 1969 — EXCEPT FOR "ORO", WHICH WAS SOWN A SECOND TIME ON APRIL 29, 1970.

Variety	Seed Yield Dz/Ha	Oil Content Percent in Dry Seed	Oil Yield Dz/Ha
Lembkes Winterraps	27.4	42.3	11.6
Line 28-2	26.1	39.6	10.3
14-5	24.9	34.3	8.5
1-6	22.2	41.5	9.2
111-1	23.7	36.6	8.7
Oro, fall sown	21.3	38.2	8.1
Oro, spring sown	16.5	37.6	6.2

SELECTION IN THE DESATURATION CHAIN OF FATTY ACID BIOSYNTHESIS

Compared to the success in the "zero erucic" breeding program, the demands for an improvement in the linoleic and linolenic acid composition (cf. Table I) were much more difficult to satisfy. The high environmental variability of this pathway is most troublesome for any genetic analysis. Everyone, who was ever active in this field, surely has something to say about disappointments. Very promising phenotypes with high linoleic and low linolenic acid content generated nothing but normal progenies. Similarly our extensive examination of more than 500 Cruciferous species and varieties did not yield the desired type. It is, however, well known, that other plant

families include species, which do not synthesize linolenic but only linoleic acid in their seed oil (cf. Thies, 1968). We therefore started mutation experiments in order to try a similar blockade in the biosynthetic pathway between the linoleic and the linolenic acid of the rapeseed.

In both mutation experiments, which have been run so far, seeds of the Canadian variety "Oro" were used. The reasoning was, that in this "zero erucic" genotype the substrate which accumulates as oleic acid before the block in the elongation chain, flows into the desaturation chain, thus enhancing the possible differences in the latter. In a pilot experiment in 1968 after 12 hrs. of soaking, 500 seeds were treated with 8 and 16 kR of X-rays and 0.2 and 0.5% EMS solutions (pH 7; 14 hrs.), respectively. In the following extensive experiment in 1969 about 5,000 M_1-plants were grown only after EMS treatment which had proven superior in mutagenic effect. So far only the first experiment has been fully evaluated. This experiment, therefore, will be described here and not only the results but also some details of the procedure will be presented, since they may be of more general interest.

In the four series of mutagenic treatments the survival varied between 45 and 90%. According to earlier experience lower rates do not increase the efficiency. The mutated M_1-plants grown from the treated seeds are expected to be largely chimeras. Therefore, 5 successive siliques were harvested from the main stem of each plant, where they are arranged in a spiral succession, thus covering a full circle around the axis. In a first test from each of the total of 1,314 M_1-plants a single M_2-seed was anlyzed for its fatty acids in the gas chromatograph. By this prescreening procedure 58 M_1-plants were selected and 42 indicated a deviating composition of their seed oil. 30-40 further seeds of these preselected M_1-plants were tested by the half-seed method. It turned out that in most cases the results of these analyses did not at all coincide with those from the first seed, from which the M_1-plants were selected; but they varied widely in any direction. Thus the single seed prescreening test proved to be largely inefficient.

Only in very few cases did the sister seeds correspond to the seeds tested first from the same M_1-plant. For example, the plant No. 778 was preselected because of the high content of both polyenoic fatty acids in the tested single seed. Later similar ratios were also recovered from all of the 22 tested M_2-sister seeds; 6 examples for these from 3 siliques (I-III) are given in Table III.

TABLE III

DEVIATING CONTENT OF 18-CARBON FATTY ACIDS IN SEEDS OF THE M_1-PLANT NO. 778 (TREATMENT 0.5% EMS): THE PROGENY OF THE PLANT M 445 MARKED WITH AN ASTERIC IS SHOWN IN TABLE IV. (SEE NOTE BELOW)

M_1-Plant No. 778	18:1	18:2	18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$
Single Seed Test	37.6	35.1	14.4	0.93	0.41
I M 438	33.2	38.1	17.3	1.15	0.45
M 439	38.0	33.8	18.3	0.89	0.54
II M 444	27.0	38.3	16.4	1.42	0.43
M 445	30.9	42.7	14.7	1.38	0.35*
III M 448	32.7	39.8	17.5	1.21	0.43
M 449	32.8	36.9	15.7	1.12	0.42
Common Rapeseed	64.3	16.8	8.0	0.26	0.48

NOTE: The data in this and the following tables give the respective fatty acids in % of the total fatty acid content.

However, all of these 22 plants produced seeds with an entirely normal fatty acid composition, which is demonstrated by a randomly chosen example in Table IV. This result agrees well with the finding of Kondra and Stefansson (1970) of maternal effects on the polyenoic fatty acid composition in rapeseeds. Apparently the M_1-plant No. 778 was physiologically affected (as an after-effect of the mutagenic treatment ?) in a way, that the composition of all of its seeds was uniformly modified and shifted towards the side of the unsaturated fatty acids.

On the other hand, our mutation experiment provided several examples, indicating that genetic deviations in the fatty acid content are more likely to be recovered in M_2-seeds showing a high variability in this characteristic. This appeared true irrespective of the fact that for the most part the analysis of the M_2-seeds was not suitable to reveal with any degree of certainty whether a mutation had occurred at all. The different possibilities of variation in the two relevant biosynthetic steps, namely, from oleic to linoleic and from linoleic to linolenic acid, were arranged into 9 groups by fixing arbitrary limits to what might be called "normal" (Table V). A total of 1,640 M_2-seeds was investigated by the half-seed method and

TABLE IV

UNCHANGED CONTENT OF 18-CARBON FATTY ACIDS IN (M_3-) SEEDS OF THE ABERRANT M_2-PLANT M 445

Progeny of 778 (M 445)	18:1	18:2	18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$
M 445-21	65.6	18.1	8.0	0.28	0.44
22	66.7	16.2	8.0	0.24	0.49
23	70.3	16.0	5.5	0.23	0.34
24	74.0	14.0	5.5	0.19	0.39
25	69.8	15.6	6.5	0.22	0.42
26	63.0	17.8	8.0	0.28	0.45
27	70.7	14.8	7.0	0.21	0.47
Common Rapeseed	64.3	16.8	8.0	0.26	0.48

639 plants grown to maturity. After self-pollination most of the progenies did not reproduce the deviation of the parental plant in the M_3-seeds. But two clear-cut results were the reward for all the expense.

TABLE V

M_2-SELECTION AFTER HALF-SEED ANALYSIS BY GROUPING ACCORDING TO 9 POSSIBLE WAYS OF VARIATION IN THE DESATURATION CHAIN. THE LIMITS FOR "NORMAL" ARE ARBITRARILY SET WITH 0.15-0.35 FOR THE QUOTIENT 18:2/18:1, AND WITH 0.3-0.7 FOR 18/3:18:2

Changes in the Desaturation Chain 18:1 → 18:2 → 18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$	Seeds Investigated
− → 0 →	<0.15	Normal	33
+ → 0 →	>0.35	Normal	375
0 → − →	Normal	<0.3	5
0 → + →	Normal	>0.7	170
− → − →	<0.15	<0.3	28
+ → + →	>0.35	>0.7	32
− → + →	<0.15	<0.7	14
+ → − →	>0.35	>0.3	5
0 → 0 →	Normal	Normal	978

In Table VI the above-mentioned uncertainty of selection among M_2-seeds from the mutagen treated M_1-plants is again evident. A low linolenic acid content was determined in the single seed pretest. None of the sister seeds, analysed by the half-seed method and grown to maturity, showed the same picture. The M_2-plant M 57 was further tested because of the low linoleic acid value in the corresponding M_2-seed. But through several generations the progeny again showed a relatively low quotient 18:3/18:2, which consistently reappeared under very different environmental conditions (Table VII). The deviation was particularly marked in the M_4-generation which was grown with continuous illumination and at a relatively high temperature (constantly 20-22°C) in the greenhouse. However, also natural length of daylight and fluctuating temperatures during the summer, which did not very much speed up the ripening processes, produced similarly low linolenic acid ratios in this mutant.

TABLE VI

CONTENT OF 18-CARBON FATTY ACIDS IN M_2-SEEDS FROM THE M_1-PLANT NO. 814 (TREATMENT 0.5% EMS)
PROGENIES OF THE PLANT M 57 ARE GIVEN IN TABLE VIII

M_1 Plant No. 814	18:1	18:2	18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$
Single Seed Test	63.7	19.8	3.6	0.31	0.18
I	65.5	16.5	9.3	0.25	0.57
	69.2	15.2	7.6	0.22	0.50
II	71.3	13.3	7.1	0.19	0.53
M 57	77.7	10.3	4.7	0.13	0.46
III	63.1	18.8	10.4	0.30	0.55
	59.5	21.5	10.1	0.36	0.47
Common Rapeseed	64.3	16.8	8.0	0.26	0.48

A similar history with regard to the origin holds true for another mutant of opposite characteristics. The M_1-plant No. 9 was selected because of a relatively high linoleic acid content of the first tested seed. The following half-seed analyses, however, showed among others some plants with a rather high amount of linolenic acid, which proved to be inheritable in the progeny of the M_2-plant M 364 (Table VIII). It should be noted, that the M_2-sister seeds show a similar ratio of 18:3/18:2, though the absolute amounts of polyenoic acids in the two seeds M 358 and M 362 vary by a factor of about 2.

TABLE VII

MEAN VALUES FOR THE CONSTANTLY DEVIATING CONTENT OF 18-CARBON FATTY ACIDS IN THE "LOW LINOLENIC" LINE M 57, GROWN IN A GREENHOUSE DURING DIFFERENT SEASONS

M 57 Generation	Seed Maturity	18:1	18:2	18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$
M_2 n=1	July 68	77.7	10.3	4.7	0.13	0.46
M_3 n=32	July 69	68.6	18.5	5.6	0.27	0.30
M_4 n=239	Jan. 70	73.3	16.2	4.1	0.22	0.25
M_5 n=131	July 70	65.4	19.5	6.8	0.30	0.35
Common Rapeseed		64.3	16.8	8.0	0.26	0.48

TABLE VIII

ORIGIN OF THE "HIGH LINOLENIC" LINE M 364 (TREATMENT 8 kR X-RAYS)

M_1 Plant No. 9		18:1	18:2	18:3	$\frac{18:2}{18:1}$	$\frac{18:3}{18:2}$
Single Seed Test		47.9	26.8	16.9	0.56	0.63
I	M 358	50.6	19.3	17.9	0.38	0.93
II	M 362	69.0	10.9	10.4	0.16	0.96
	M 364	54.4	15.1	20.4	0.28	1.34[A]
	M 364-734	50.7	20.7	20.7	0.41	1.00
M_3 Seeds	744	50.8	17.6	23.3	0.35	1.32
	754	50.7	22.0	19.8	0.43	0.91
Common Rapeseed		64.3	16.8	8.0	0.26	0.48

To further prove the inheritability of the demonstrated differences within the desaturation chain, the "low linolenic" line M 57 and the "high linolenic" line M 364 were reciprocally crossed (Figure V). The F_1's, i.e. the seeds obtained by crossing, clearly showed intermediate ratios of the fatty acids in question. From Figure V no significant maternal effect is evident from the reciprocal combinations. The F_2-seeds, therefore, exhibit a distinct segregation (Figure VI). Because of the almost continuous variation of the F_2-population a genetic analysis of the F_3 is now underway. So far it appears possible, that the genetic basis of the observed difference is more complex and simple genetic ratios will not be received.

In summary we have to admit, that the desired complete blockade in the desaturation chain between the linoleic and the linolenic acid has not been found as yet. There are indications that the linolenic acid is an essential cell constituent, wherever the developing embryo becomes green during development before seed maturity. This is the case in Crucifers but not in plants like sunflower, cotton or peanut, which entirely lack linolenic acid in their seeds (Thies 1970)(a).

Nevertheless, at least some genetic variations in the 18:3/18:2 ratio are possible. It remains to be established, whether more drastic mutations of the kind detected above, can be found by further screening, or whether similar, but genetically different small steps can be joined by recombination in order to increase the linoleic and decrease the linolenic acid in rapeseed to the furthest possible extent. We hope that progress can be made in the near future by screening more material of our mutation experiments by more efficient analytical methods, which will allow a photometric determination of the linoleic and linolenic acids after alkali-isomerization (Thies 1970)(b).

FIGURE V

CONTENT OF THE 18-CARBON FATTY ACIDS IN THE "LOW LINOLENIC" MUTANT M 57 AND THE "HIGH LINOLENIC" MUTANT M 364 AND IN RECIPROCAL F_1'S BETWEEN THEM

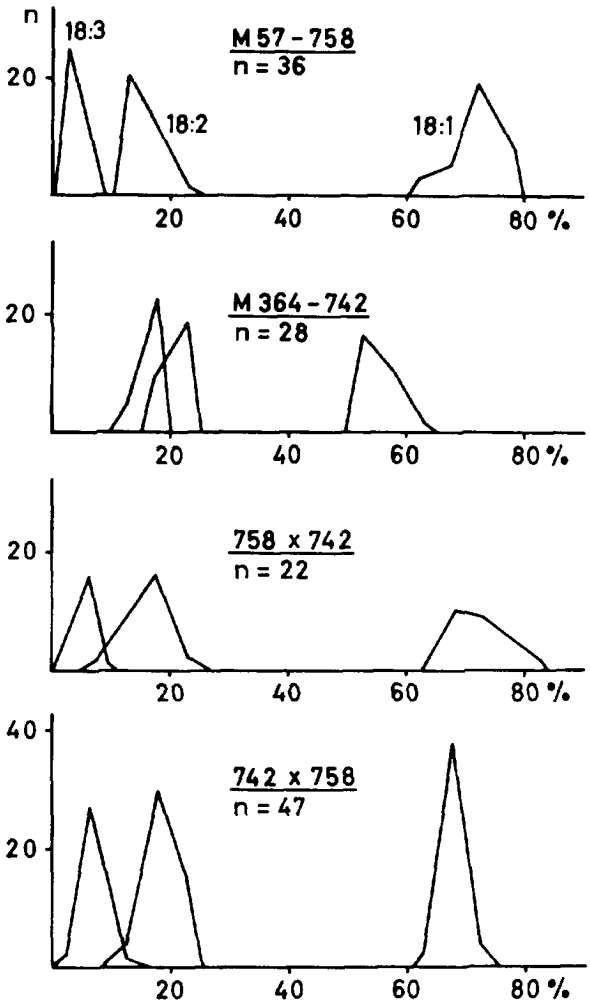
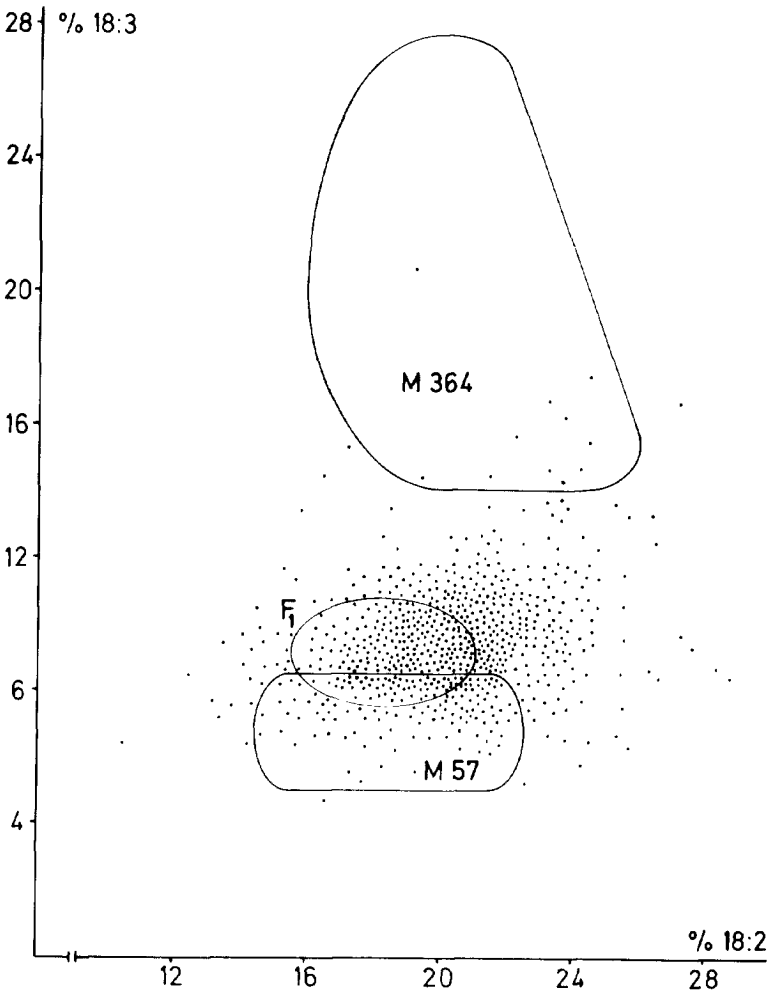


FIGURE VI

SEGREATION IN 688 F_2-HALF-SEEDS (EACH SHOWN AS ONE DOT) IN RESPECT TO THEIR COMPOSITION OF POLYENOIC FATTY ACIDS COMPARED TO THE AREA OF THE CORRESPONDING VALUES FOR BOTH PARENTAL LINES, M 364 AND M 57, AND THE F_1-SEEDS. ALL PLANTS WERE GROWN UNDER THE SAME CONDITIONS; THE RECIPROCAL PRODUCTS OF THE CROSSES WERE POOLED. NOTICE THE MUCH LARGER VARIATION IN THE "HIGH LINOLENIC" LINE M 364!



LITERATURE REFERENCES

Downey, R.K.: Oil quality in rapeseed. Can. Food Industry, June 1963.

Downey, R.K. and B.L. Harvey: Methods of breeding for oil quality in rape. Canada J. Agric. Sci. 43, 271-275 (1963).

Kondra, Z.P. and B.R. Stefansson: A maternal effect on the fatty acid composition of rapeseed oil (Brassica napus). Can. J. Plant Sci. 50, 345-346 (1970).

Riemann, K.H. and H. Kruger: Untersuchungen uber Möglichkeiten zur Frühselektion auf hohen Fettgehalt bei Winterraps. Züchter 37, 226-231 (1967).

Stefansson, B.R. and F.W. Hougen: Selection of rape plants (Brassica napus) with seed oil practically free from erucic acid. Can. J. Plant Sci. 44, 359-364 (1964).

Stefansson, B.R., F.W. Hougen and R.K. Downey: The isolation of rape plants with seed oil free from erucic acid. Can. J. Plant Sci. 41, 218-219 (1961).

Thies, W.: Die Biogenese von Linol- und Linolensäure in den Samen Höherer Pflanzen, insbesondere Raps- und Rübsen, als Problem der Ölpflanzenzuchtung. Angew. Bot. 42, 140-154 (1968).

Thies, W.: Der Einfluß der Chloroplasten auf die Bildung von ungesättigten Fettsäuren in reifenden Rapssamen. Fette, Seifen, Anstrichmittel (in the press) (1970).(a)

Thies, W.: Rapid and simple techniques for the determination of the fatty acid composition in single cotyledons of rapeseed. Z. Pflanzenzüchtung (in preparation) (1970).(b)