# CHLOROPLAST DEVELOPMENT AND BIOGENESIS OF LINOLENIC ACID IN RIPENING COTYLEDONS OF RAPESEED

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Among the quality requirements that the oil processors set for the rapeseed oil a reduced level of linolenic acid will surely range first in the long run. Whereas the reduction of the erucic acid content even to the zero level has presented no major difficulties to the plant breeders, the question of the linolenic acid has remained unsolved until today. In the following I will try to discuss some aspects that may point towards the background of this problem.

If the conditions found in oilseeds from very different plant families are reviewed, the following correlation can be recognized:

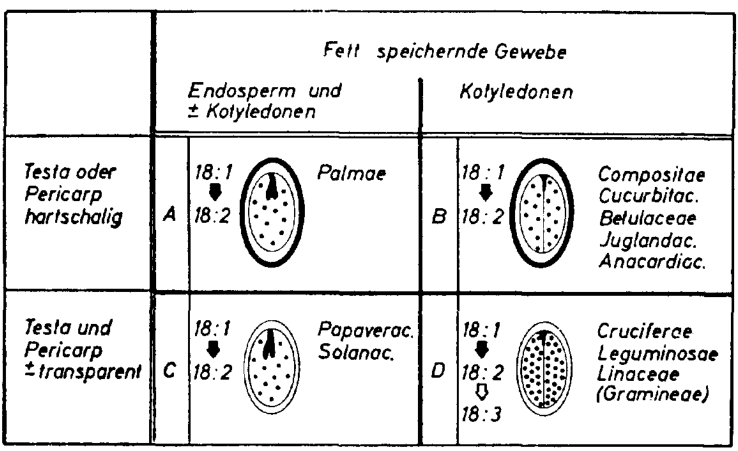


FIGURE I

THE FOUR MAIN TYPES OF THE FAT STORING ANGIOSPERM SEEDS; ONLY THE FAMILIES NAMED IN GROUP D SYNTHESIZE LINOLENIC ACID IN THEIR SEEDS BESIDES OLEIC AND LINOLEIC ACID.

In general the main storage tissues for seed oils are either the cotyledons (Figure 1, group B and D) or the endosperm (group A and C). In some families the fruits develop a hard pericarp (group A and B). In other cases fruit and seed walls are more or less penetrable to light (group C and D). Seeds of all groups are able to produce oleic and linoleic acid. But the accumulation of linolenic acid occurs only in seeds, which at least during certain stages of their development, possess green and photosynthetically active chloroplasts. The Cruciferous plants unfortunately belong to the latter group. Screening analyses performed in our laboratory on 540 species and cultivars of the Cruciferae family revealed that probably all plants belonging to this family contain smaller or larger amounts of linolenic acid in their seed oil.

This obviously close correlation between chloroplast development and accumulation of linolenic acid in ripening seeds was strikingly confirmed by a great number of investigations during the greening of etiolated plants or on growing and aging green tissues (1). Except for this physiological and morphological correspondence, functional correlations have also been shown. It is obvious from several examples, that the photosynthetic activity of chloroplasts, measured by the oxygen produced, corresponds to the different levels of linolenic acid found in the chloroplasts during development and aging (2). If one counts on the assumption that the photosynthetically produced oxygen has a favourable effect on the biogenesis of the linolenic acid (1), a process like an autocatalytic reaction could be visualized: The developing chloroplast produces increasing amounts of oxygen; these enhance the desaturation of the linoleic acid; the latter improves the effectiveness of the photosynthetic apparatus and so on. Whatever the specific mechanism responsible for the accumulation of the linolenic acid in green tissues may be, there is ample evidence for the statement, that the linolenic acid plays an essential role in the photosynthetic process either functionally or as an important buildingstone of the chloroplast membranes. In contrast to the erucic acid, which can be regarded as a typical storage fatty acid with probably no other functions within the cells, the linolenic acid represents a physiologically highly active component, which cannot be eliminated from the seed without severe consequences.

To prove the above-mentioned correlations of the stage of chloroplast development and the biogenesis of the linolenic acid, two strains were selected, one with a linolenic acid content of 4-7% and another with 15-20% in the seed oil. These strains were derived from a mutation experiment after treatment of seeds of the Canadian "zero erucic" variety "Oro" and they were shown to be genetically stable during four inbred generations. Flowers from both strains were selfpollinated and the developing seeds were harvested in weekly intervals. Analyses were made of the total oil, the total nitrogen content and the fatty acid composition; and the chlorophyll content was determined as a measure of the chloroplast development.

It is evident from Figure 2, that the chlorophyll content of the two strains differed during all stages of development. The strain M 57 reached the maximum greening of the seeds after 24 days, and 45 days after pollination almost all chlorophyll was decomposed. The strain M 364 developed much more chlorophyll with a maximum at the 31st day and the breakdown was nearly complete only after 52 days. The chlorophyll content based on the total oil weight and the corresponding proportions of the two polyunsaturated fatty acids during seed development are given in Figure 3. The shape of the chlorophyll curves is essentially the same as in the preceding figure. As for the level of the fatty acids, the M 57 strain resembles normal "zero erucic" (3). After relatively high values for the two acids in the first stages of development, their proportions drop slowly until the final percentages are reached in the mature seeds. In contrast, the mode of the linolenic acid biogenesis in strain M 364 is most remarkable. Within the first three weeks the linolenic acid seems to follow the tendency observed usually. But between the 25th and 45th day an increased production of this acid can be noticed. During this time the chlorophyll content in the seeds of strain M 364 is much higher than is true for the strain M 57.

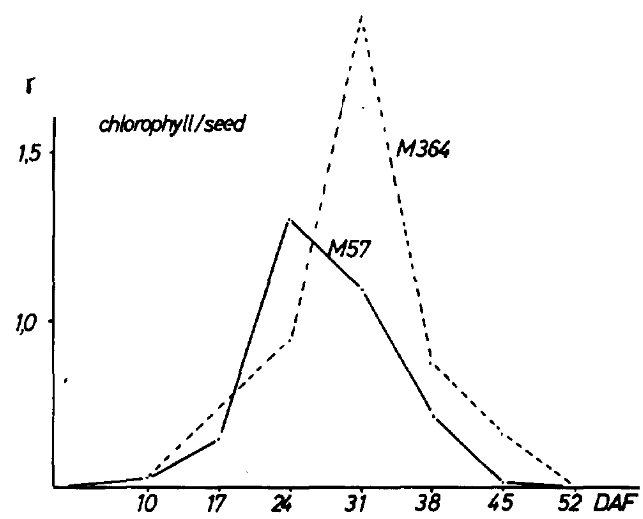


FIGURE II

CHLOROPHYLL CONTENT OF MATURING SEEDS FROM STRAINS M 57 AND M 364 (DAF = DAYS AFTER FLOWERING)

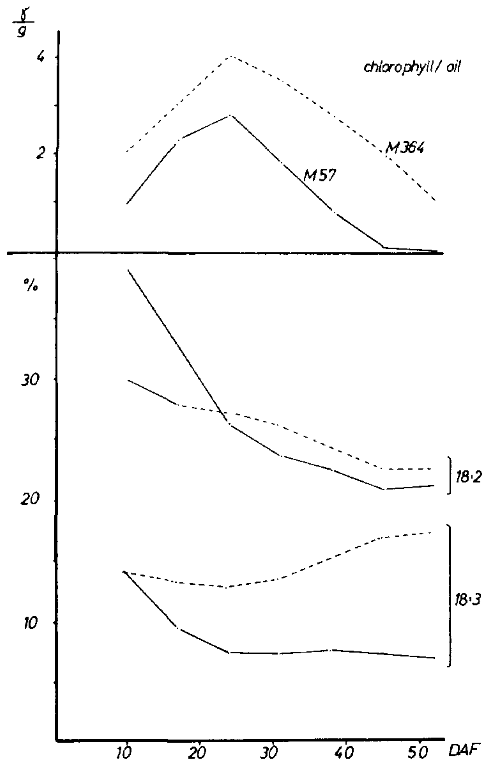


FIGURE III

CHLOROPHYLL AND FATTY ACID CONTENTS OF MATURING SEEDS FROM STRAINS M 57 AND M 364 BASED ON OIL WEIGHT (DAF = DAYS AFTER FLOWERING)

If we assume, that the higher chlorophyll content indicates a higher photosynthetic activity, higher values of the total oil and total nitrogen content should be expected. According to Figure 4 this is indeed true. Compared with strain M 57 the oil accumulation in seeds from strain M 364 occurs with some delay in the first stages of ripening, but reaches higher values 30 days after pollination.

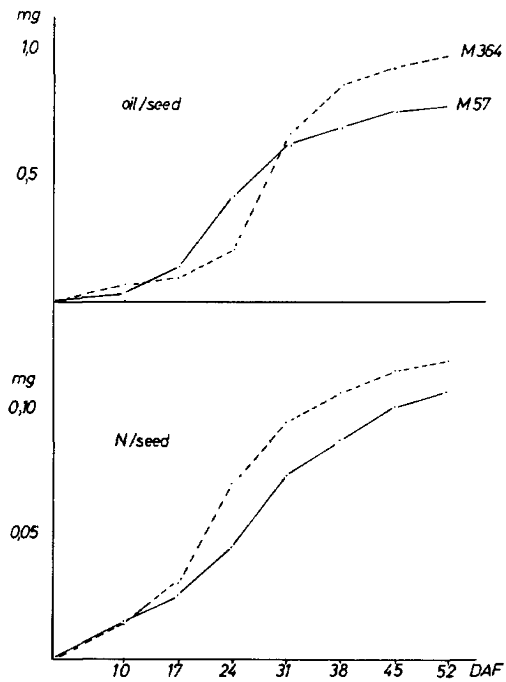


FIGURE IV

OIL WEIGHT AND WEIGHT OF TOTAL NITROGEN OF MATURING SEEDS FROM STRAINS M 57 AND M 364 (DAF = DAYS AFTER FLOWERING)

It is well-known, that the biogenesis of the polyunsaturated fatty acids is influenced by environmental conditions, such as light and temperature. But also the age of the plant may play an important role. We observed marked differences in the content of linoleic and linolenic acid as well as chlorophyll in seeds from the same plant ripening at an interval of only two weeks. In a first experiment, flowers were pollinated on June 19 (Figure 5, upper part) and in a second one 14 days later (lower part). The first column of the figure stands for the maximum chlorophyll content (in ppm based on oil content), which was attained during seed development. Values for the fatty acids were measured at the stage of maturity. In the first experiment both strains showed higher values for all compounds mentioned. It is remarkable, however, that the relative proportions of the two fatty acids were the same in both experiments. The quotient $\frac{\% \; 18:3}{\% \; 18:2}$ for strain M 57 remained 0.34 and that for M 364 nearly 0.16.

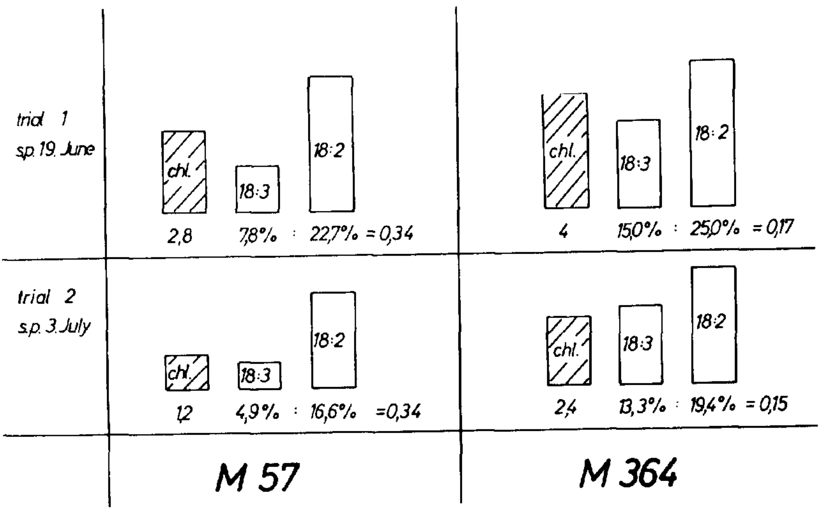


FIGURE V

CHLOROPHYLL (PPM BASED ON OIL WEIGHT) AND FATTY ACID (%) CONTENT OF SEEDS FROM THE SAME PLANT OF STRAINS M 57 AND M 364, RIPENING AT AN INTERVAL OF TWO WEEKS.

In summary, it can be stated that at least in the two strains used for the experiments the chloroplasts in the ripening seeds seem to exert a marked influence on the biogenesis of the poly-unsaturated fatty acids as well as on the total amount of oil. The higher the chlorophyll content and the longer it is present in measurable amounts, the greater is the amount of linolenic acid found in the mature seeds. In the seeds of strain M 57 a lower amount and an earlier decomposition of the chlorophyll is correlated with desirably low values for the linolenic acid; but unfortunately the total weight of oil is also reduced.

There is no doubt that the chloroplasts of the seeds play an important role in the energy and carbon supply of the growing embryo. From electron microscopic pictures it can be seen that in all stages of seed ripening the chloroplasts include starch grains. Darkened siliques, on the other hand, produce only one third of the oil normally found in illuminated ones (1). Therefore plants with well developed chloroplasts are expected to compete with advantage under conditions of natural selection. This may be the reason that all seeds analysed so far from Cruciferous plants contain green cotyledons. There is furthermore no doubt that a well working chloroplast - as mentioned at the beginning - is characterized by a high content of linolenic acid. Accordingly in Table 1 the fatty acid composition in chloroplasts of plants from a higher evolutionary stage indicates a tendency to increased linolenic acid accumulation.

TABLE I

FATTY ACID COMPOSITION IN CHLOROPLASTS OF MOSSES, FERNS, GYMNOSPERMS AND ANGIOSPERMS

| | FATTY ACIDS IN CHLOROPLASTS | |
|---|---|---|
| | 18:2 | 18:3 |
| Mosses | 9-15 | 10-22 |
| Ferns | 15-31 | 9-42 |
| Gymnosperms | 14-20 | 24-46 |
| Angiosperms | 8-21 | 23-71 |

The correlation observed between chlorophyll and linolenic acid content suggests the possibility of a positive influence of the photosynthetic oxygen on the biogenesis of this fatty acid. But it has to be kept in mind that the biogenetic processes are generally connected with other metabolic reactions. Thus, different mechanisms of oxidation, including β-oxidation or action of lipoxygenases, possibly regulated by natural antioxidants, may determine the final fatty acid composition.

From Table 2 it can be seen that the variability of the polyunsaturated fatty acids among the Cruciferae is quite large. Malcolmia litorea, with a fatty acid content resembling linseed oil, may be compared to Conringia orientalis with a fatty acid composition similar to that of groundnut oil. Whatever the factors may be, which establish the individual fatty acid pattern, the mere existence among the Cruciferae of representatives with very high and very low levels of linolenic acid in the seed oil should encourage further search for mutants with the desired seed oil composition.

TABLE II

VARIABILITY OF THE POLYUNSATURATED FATTY ACIDS AMONG THE CRUCIFERAE

| % 18:2 | : | % 18:3 | | % 18:2 | % 18:3 |
|---|---|---|---|---|---|
| 1 | : | 5 | Malcolmia litorea | 13 | 66 |
| 1 | : | 4 | Aethionema grandiflorum | 15 | 62 |
| | | | Alyssium montanum | 14 | 60 |
| 1 | : | 3 | Hutchinsia auerswaldi | 13 | 39 |
| 1 | : | 2 | Bunias orientalis | 24 | 52 |
| | | | Camelina microcarpa | 25 | 45 |
| 1 | : | 1 | Cakile maritima | 15 | 17 |
| | | | Cardaminopsis halleri | 15 | 14 |
| 2 | : | 1 | Barbarea vulgaris | 25 | 13 |
| | | | Cochlearia glastifolia | 25 | 14 |
| 3 | : | 1 | Lumaria rediviva | 21 | 7 |
| | | | Iberis umbellata | 19 | 6 |
| 4 | : | 1 | Sisymbrium loeselii | 35 | 9 |
| 10-15 | : | 1 | Conringia orientalis | 29 | 2.3 |
| | | | Rorippa nasturtium-aqu. | 19 | 1.7 |

LITERATURE REFERENCES

(1): W. Thies: Der Einfluss der Chloroplasten auf die Bildung von ungesattigten Fettsauren in reifenden Rapssamen; Fette, Seifen, Anstrichmittel, in press.

(2): T.E. Weier, A.A. Benson: The Molecular Nature of Chloroplast Membranes, in: T.W. Goodwin (ed): Biochemistry of Chloroplasts Vol. I, 91-113, Academic Press, New York, 1966.

(3): D.B. Fowler, R.K. Downey: Lipid and Morphological Changes in Developing Rapeseed, Brassica napus; Can.J.Plant Sci. 50 : 233-247, 1970.

COMMENTS: (Dr. R.K. Downey)

1) I just want to make one comment here. I think we will soon be able to prove your thesis one way or another, in that my colleague Mr. Stringham, now has *Brassica campestris* without chlorophyll in the cotyledon, it is a genetic mutant and we have both chlorophyll-free and chlorophyll-containing seeds within the same pod. Within two days we will know.

COMMENTS: (Dr. R.G. Robbelen)

2) I may just answer Dr. Downey. We did this a year ago, using albino mutants. This, unfortunately, does not entirely give the answer. There was surely a reduced content in linolenic acid, but it is not as much as we might expect, when things are directly and only dependent on the chloroplast. There seems to be some other factors involved.