Genotypic differences between the contents of linolenic acid in galactolipids and triglyceride from seeds of rape plants

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Introduction

For nutritional and technological reasons linolenic acid (18:3) is undesirable in rape-seed oil. However, progress in selection for low 18:3 within the C_{18} fatty acids has been essentially slower than in the case of breeding for zero erucic cultivars as was recently reviewed by Röbbelen (1982). Rakow (1973) discovered the first induced mutant (M_{57}) by treatments with X-rays or EMS. As compared to the original form 'Oro' (10 %), M_{57} showed a reduced level of linolenic acid (5.5 %). Using the same method, further improvement was achieved by Röbbelen und Nitsch (1975) who selected mutants with less than 3.5 % linolenic acid.
Total elimination of linolenic acid from seed oil may not be possible. With respect to this presumption, a strong correlation between the occurrence of chloroplasts and the formation of linolenic acid in the developing seed has been stressed by Thies (1971). Nevertheless, the principal functions of plastids for linolenic acid synthesis in the storage fraction are not yet understood.
The present paper deals with a physiological study concerning the formation of linolenic acid in galactolipids and triglyceride from the 'Oro' cultivar and its mutants with different polyenoic fatty acid contents. Generally, galactolipids are accepted to be typical lipids of chloroplast thylakoids while triglycerides represent the storage fraction. The question is still unsolved whether there exist similar patterns of linolenic acid formation in the two lipid classes.

Material and Methods

Plants of the mutants M_{57} (about 5 % linolenic acid) and M_{364} (about 20 % linolenic acid) originally described by Rakow (1973) and plants of the 'Oro' cultivar (about 10 % linolenic acid) were grown under semi-controlled greenhouse conditions. In order to fix the exact physiological age of seeds buds from terminal racemes were self-pollinated by hand. 500 seeds per genotype were harvested at 10, 20, 30, 40 and 50 days after flowering (DAF).
Lipids were extracted with chloroform-methanol-water (8:4:3 by vol) in the presence of 0.2 % butylated hydroxytoluene as an antioxidant. Triglycerides (TG), monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) were separated by thin layer chromatography. Saponification of lipids and methylation of fatty acids were carried out with methanolic NaOH and BF_3-methanol and the fatty acid methyl esters were determined by gas chromatography (Diepenbrock, 1981).
A one-way analysis of variance was performed on three treatments (M_{57}, M_{364}, Oro) and four replications per treatment. Pair comparisons among treatment means were carried out using the LSD with an α level of 5 %.

Results

Table 1 shows the coefficients of correlation between %18:3 in MGDG and DGDG and %18:3 in TG at five sampling dates. The contents of 18:3 in galactolipids at 10 DAF and those in TG were not significantly correlated. The percentage of 18:3 in MGDG at 20 DAF was closely related to the values in TG at the same date while thereafter, until maturity, the correlations weakened. No significant correlation occurred between %18:3 in DGDG at day 20 and %18:3 in TG. However, significant values were found at days 30 and 40, resp..

Tab. 1 Coefficients of correlation between the linolenic acid contents (%18:3) of monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) and the linolenic acid contents (%18:3) of triglyceride (TG)

		%18:3 MGDG						%18:3 DGDG					
		10	20	30	40	50	DAF	10	20	30	40	50	DAF
	DAF												
	10	+0,30						-0,20					
	20	+0,06	+0,82					-0,07	+0,32				
%18:3 TG	30	-0,13	+0,56	+0,62				+0,04	+0,39	+0,73			
	40	+0,03	+0,63	+0,61	+0,54			+0,11	+0,32	+0,74	+0,70		
	50	-0,08	+0,68	+0,71	+0,70	+0,62		-0,09	+0,39	+0,85	+0,83	+0,60	

$r_{05} = 0,40$

Figure 1 elucidates the changes in the amounts of linolenic acid (µg 18:3) in TG, MGDG and DGDG during the whole course of seed development. From 10 DAF to 20 DAF, only a small increase was proved for all genotypes in TG. During the following 10 days, seeds of Oro revealed a comparatively high rate of 18:3 accumulation.
Thereafter, both Oro and M_{57} were marked by a slow rate of increase. In contrast, seeds of M_{364} attained the highest rate from day 30 to day 40. At maturity, the 18:3 deposition in seeds of M_{364} exceeded that of the other genotypes.
The 18:3 accumulation in galactolipids was quite different from that of TG. Starting from a low level at 10 DAF, the highest values were reached at days 20 and 30. Within 10 days thereafter a dramatical decrease was observed continuing until maturity. The most intensive 18:3 accumulation occurred in seeds of Oro. At day 20, Oro and M_{364} had appreciably high amounts of 18:3 in both galactolipids; whereas for M_{57}, maximum values were measured at 30 DAF. No genotypic differences appeared with regard to the sharp decrease in 18:3 weights from day 40 to day 50.

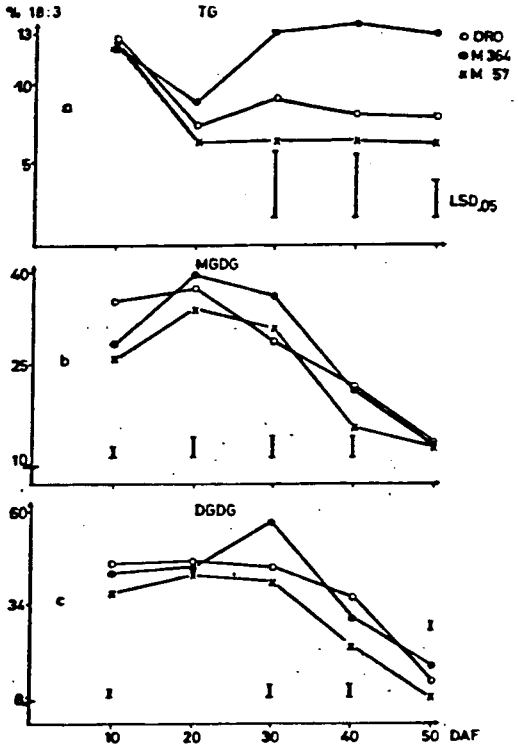

Fig. 1 Amounts of linolenic acid (µg 18:3) per seed in triglyceride (TG), monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) during seed development

The percentage of 18:3 usually represents a certain measure for the specific ability to synthesize 18:3 as compared to other fatty acids. Generally, the 18:3 contents in TG were high at 10 DAF and then fell off to a minimum at 20 DAF (Fig. 2). In seeds of M_{57} the 18:3 level then remained unchanged until 50 DAF. However, in seeds of both other genotypes an increase from 20 DAF to 30 DAF was observed. At maturity M_{364} is marked overall by the highest 18:3 content in TG followed by Oro and M_{57}. As was already demonstrated for the amounts of 18:3 in galactolipids the percentages also developed in an optimum curve. High levels were gained by M_{364}. In the first instance, this became obvious for DGDG at day 30. Comparing both mutants, it was found that the values for M_{364} were throughout higher than those for M_{57}.

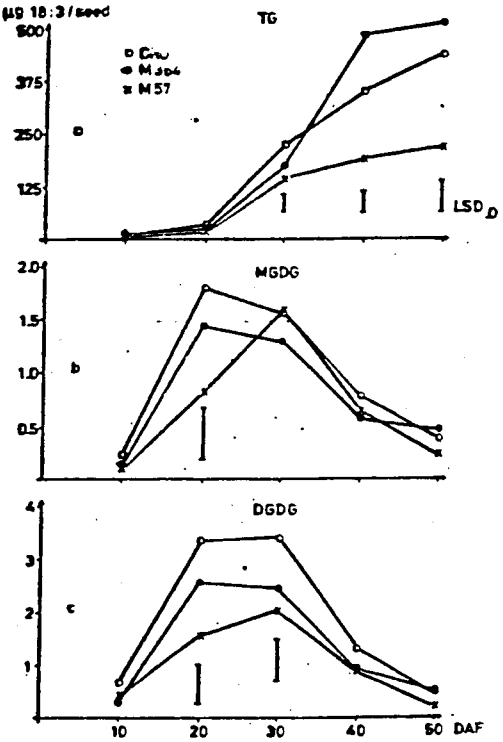

Fig. 2 Percentages of linolenic acid (%18:3) in triglyceride (TG), monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) during seed development

Discussion

According to the hypothesis of Harris and James (1969), Thies (1971) suggested that the D_2 development by chloroplasts controls the formation of linolenic acid in rape-seed triglyceride in an unspecific manner. However, the present results seem to contradict this presumption. A comparison between galactolipids (typical lipids of chloroplasts) and triglyceride (storage lipids in the cytoplasm) pointed towards different regulations for linolenic acid synthesis in the two lipid classes. This is in line with the principal finding that chloroplasts possess an independent system for de-novosynthesis of fatty acids (Roughan et al., 1979). On the other hand, the biosynthetic activity of plastids could not have provided 18:3 for triglyceride since the deterioration of chloroplasts - indicated by decreasing amounts of 18:3 in galactolipids - started at about 30 DAF. During the same period the most intensive 18:3 accumulation in triglyceride occurred. Nevertheless, the present results (Tab. 1) indicated significant correlations between %18:3 of galactolipids and %18:3 of triglyceride. Furthermore, [illegible]s mutant with a high percentage of 18:3 in triglyceride also revealed high values in typical chloroplast lipids and vice versa (Fig. 2). Hence, the question arises whether there exists a common regulatory process. In this connection it should be noted that 18:1-phosphatidyl choline is precursor for desaturation of triglyceride in cotyledons of oil-bearing seeds (Dybing and Craig 197/; Slack et al., 1978) and of chloroplast membrane lipids (Drapier et al. 1982; Stymne and Appelqvist, 1978; Tanaka et al., 1980). Hence, the close correlation between %18:3 of the two lipid classes could be explained by a common precursor for desaturation in different compartments of the cell. On this basis a genom/plastom-interaction might be possible. In order to test this hypothesis, reciprocal crosses between 18:3-mutants should be carried out. Tilney-Bassett and Abdel-Wahab (1979) showed, for some brassicaceae, that plastom information is not transmitted through pollen. Hence, the cross 'low 18:3 x high 18:3' should result in a F_1 with a 18:3 content below the theoretical average. In contrast, the reciprocal cross should provide a higher percentage of 18:3 than the average of the F_1's.

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Literature

Diepenbrock, W.: Effects of light, temperature and nitrogen treatments upon the fatty acid composition of galactolipids of young and older leaves from winter rape plants. Physiol.Plant. 52, 1-6, 1981.

Drapier, D.; Dubacq, J.-P.; Trémolières, A. and Mazliak, P.: Cooperative pathway for lipid biosynthesis in young pea leaves: oleate exportation from chloroplasts and subsequent integration into complex lipids of added microsomes. Plant Cell Physiol. 23, 125-135, 1982.

Dybing, C.D. and Craig, B.M.: Fatty acid biosynthesis and incorporation into lipid classes in seeds and seed tissues of flax. Lipids 5, 422-429, 1970.

Harris, P. and James, A.T.: Effect of low temperature on fatty acid biosynthesis in seeds. Biochim.Biophys.Acta 187, 13-18, 1969.

Rakow, G.: Selektion auf Linol- und Linolensäuregehalt in Rapssamen nach mutagener Behandlung. Z.Pfl.zücht. 69, 62-82, 1973.

Röbbelen, G.: Changes and limitations of breeding for improved polyenoic fatty acid content in rapeseed. AOCS Meet., Toronto, 1982.

Röbbelen, G. and Nitsch, A.: Genetical and physiological investigations on mutants for polyenoic fatty acids in rapeseed, Brassica napus L.. Z.Pfl.zücht. 75, 93-105, 1975.

Roughan, P.G.; Holland, R. und Slack, C.R.: The role of chloroplasts and microsomal fractions in polar-lipid synthesis from [1-^{14}C] acetate by all free preparations from spinach (Spinacia oleracea) leaves. Biochem.J. 188, 17-24, 1979.

Slack, C.R.; Roughan, P.G. and Balasingham, N.: Labeling of glycerolipids in the cotyledons of developing oilseeds by [1-^{14}C] acetate and [2-^{3}H] glycerol. Biochem.J. 170, 421-433, 1978.

Stymne, S. and Appelqvist, L.-A.: The biosynthesis of linoleate from oleoye-CoA via oleoye-phosphatidyl-choline in microsomes of developing safflower seeds. Eur.J.Biochem. 90, 223-229, 1978.

Tanaka, T.; Ohnishi, J. and Yamada, M.: The occurrence of phosphatidyl-choline exchange protein in leaves. Biochem.Biophys.Res.Commun. 96, 394-399, 1980.

Thies, W.: Der Einfluß der Chloroplasten auf die Bildung von ungesättigten Fettsäuren in reifenden Rapssamen. Fette, Seif., Anstr. 73, 710-715, 1971.

Tilney-Bassett, R.A.E. and Abdel-Wahab, O.A.L.: Material effects and plastid inheritance. In: Maternal Effects in Development. Eds. D.R.Newth and M.Balls. Brit.Soc.Develop.Biol.Sympos. 4, 29-45, 1979.