INHERITANCE OF LINOLENIC ACID ESTERIFIED TO GALACTOSYL DIACYLGLYCEROL AND TRIACYLGLYCEROL FROM SPECIFIC RAPESEED (BRASSICA NAPUS L.) MUTANTS

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INTRODUCTION

Rapeseed (Brassica napus L.) is an amphidiploid containing the genomes of B. oleracea and B. campestris. Generally, the seed oil of all Brassica species is distinguished by a high concentration of erucic acid (22:1). Most studies on the genetic regulation of fatty acid composition in rapeseed have dealt with 22:1 because ingestion of 22:1 may lead to reduced growth and undesirable physiological changes in animals. These studies have revealed that 22:1 concentration was conditioned by multiple alleles at two loci in rapeseed and one locus in turnip rape (Jönsson 1977). It was also observed that the genotype of the developing embryo controlled 22:1 composition of rapeseed. Embryonic control rather than strictly maternal influence on 22:1 plus simple Mendelian inheritance of 22:1 expedited breeding progress toward the development of zero-22:1 germplasm. Because 22:1 concentration was determined by the genotype of the seed, effective selections could be made in the F_2 generation.

Like soybean, rapeseed oil also contains relatively high levels of 18:3 (ca. 9 % w/w). 18:3 is undesirable because its three doublebonds are sensitive to oxidation during processing. Although genetic variability for 18:3 concentration in crucifers is low (This 1968), Rakow (1973) and Röbbelen and Nitsch (1975) have induced mutants that range from 2 - 20 % 18:3. However, the success of a breeding program for low 18:3 rapeseed may be dependent upon the mode of 18:3 inheritance in such mutants (Diepenbrock 1983).

The basic understanding of fatty acid synthesis in plant tissue has recently been improved due to progress in elucidating the cellular locations of the biosynthetic pathways involved. There is now evidence that de novo fatty acid synthesis occurs almost exclusively in the plastids. Thies (1971) emphasized that the content of 18:3 in seed oils is correlated with the occurrence of chloroplasts during seed development. Nevertheless, the principal functions of plastids for 18:3 synthesis in the storage fraction are not yet understood. For breeding purposes the question arose whether genetic information from the plastome would partially control the 18:3 content in rapeseed oil.

Therefore, the present study was undertaken to evaluate reciprocal effects on 18:3 content in galactolipids which are commonly known as typical lipids of chloroplast thylakoids and also on 18:3 content in triacylglycerol. Genetic diversity was introduced by using induced mutants for polyenoic fatty acids M_{43} (about 3 % 18:3) and M_{364} (about 14 % 18:3) which were kindly provided by Prof. Röbbelen, Göttingen. Reciprocal crosses were made between these mutants. The resultant F_1 progenies also were reciprocally backcrossed to the original parents in all possible two-way combinations. Each treatment, including self-pollinated parents, were replicated four times in a complete block design. Four subsamples of seed from each replication were harvested at 30 and 50 days after flowering (DAF) and prepared for lipid extraction.

RESULTS AND DISCUSSION

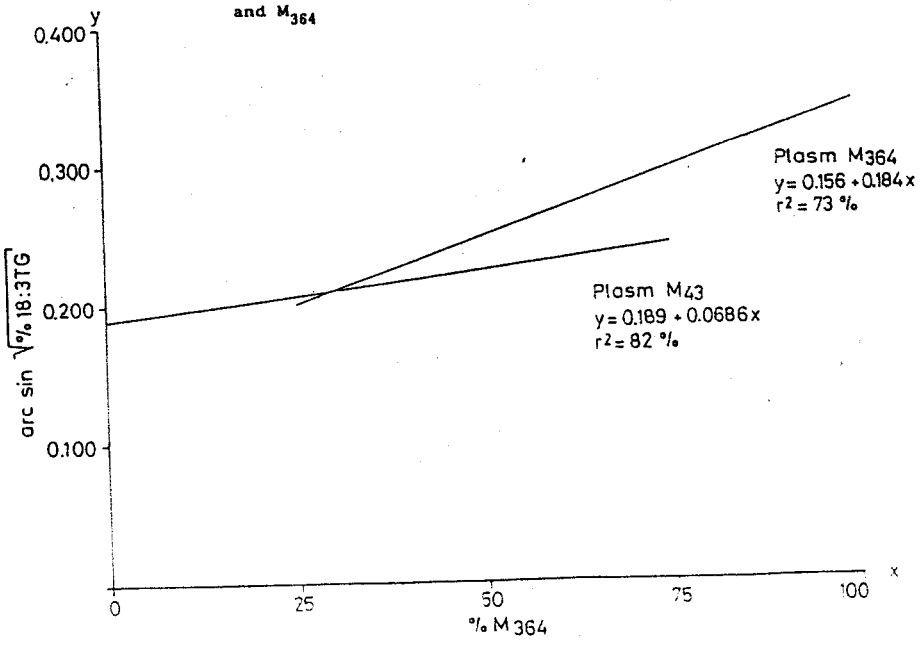
Examination of the data for TG shown in Table 1 revealed that the 18:3 concentration of the F_1 progenies of the reciprocal crosses between M_{43} and M_{364} was significantly different from each other and also was different from the maternal parent. There was no clear indication of an exclusive maternal or paternal influence on 18:3 concentration in these data, but both parents appearently exerted some degree of influence on 18:3. In contrast to these data for TG, 18:3 concentration in MGDG was determined by the maternal parent in all sets of crosses. Within each set, 18:3 in the reciprocal backcross was not

TABLE 1 EVALUATION OF CYTOPLASMIC EFFECTS UPON LINOLENIC ACID CONCENTRATION IN MUTANTS OF RAPESEED (according to DIEPENBROCK & WILSON 1987)

Cytoplasm	Genotype		Cross	Genotype	Phenotype	
	Maternal plant	Pollen Donor			*TG (50 DAF)	*MGDG (30 DAF)
				% "B"	% 18:3	
bb	bb	bb	M43 x M43	0	3.3	33.0
	bb	(bbxBB)	M43 x (M43 x M364)	25	3.8	35.0
	bb	(BBxbb)	M43 x (M364 x M43)	25	5.2	32.1
	(bbxBB)	bb	(M43 x M364) x M43	25	4.2	30.3
	bb	bb	M43 x M364	50	5.0	30.1
	(bbxBB)	BB	(M43 x M364) x M364	75	6.5	32.1
BB	(BBxbb)	bb	(M364 x M43) x M43	25	4.7	41.0
	bb	bb	M364 x M43	50	6.8	47.1
	BB	(bbxBB)	M364 x (M43 x M364)	75	7.8	45.8
	BB	(BBxbb)	M364 x (M364 x M43)	75	7.2	45.3
	(BBxbb)	BB	(M364 x M43) x M364	75	7.5	47.5
	BB	BB	M364 x M364	100	8.8	47.9

*TG, triacylglycerol; MGDG, monogalactosyldiacylglycerol

Figure 1. Regression of M_{364} genom (% M_{364}) on linolenic acid (% 18:3) contents in triglyceride (TG) at 30 DAF for plasms of M_{43} and M_{364}



different from the maternal plant. Differences between the respective reciprocal backcrosses occurred only when the genotype of the cytoplasm was different. In cases when the maternal plants of the reciprocal backcrosses were genotypically different, but contained identical cytoplasm, there were only slight differences in 18:3. Therefore it was highly probable that 18:3 in MGDG was mainly determined by a cytoplasmic influence.

For furtner genetical analysis of 18:3 inheritance in TG, linear regressions were calculated for sets according to either M_{43} plasm or M_{364} plasm at 30 DAF (Fig. 1). Proportions of M_{364} genome (% M_{364}) within the crosses and arc sin $\sqrt{\%\ 18:3\ TG}$ represented the independent and dependent variables. Principally, the additive constant indicates a plasmic influence whereas the slope indicates a genome effect. The additive constants as well as the regression coefficients deviated significantly from zero. Moreover, a comparison of the regression lines resulted in highly significant differences between the two plasms. An increase of % M_{364} caused different responses of 18:3 contents depending on the plasmic constitution. Consequently, the difference in slope of the lines proved an interaction between plasmic and genome effects.

Thies (1971) suggested that the relatively high amount of 18:3 in seed oils of Brassica species was a consequence of the early green stages of the developing embryo. Seed growth from about 15 to 30 DAF was the most active interval in the accumulation of storage lipids (Diepenbrock and Geisler 1979). During this period chloroplasts completed their differentiation and consequently, measurements of gas exchange showed a peak of CO_2 uptake by isolated seeds at this stage (Diepenbrock 1984). Hence, it appeared to be highly important to elucidate the relevance of plastidic activity for 18:3 synthesis in storage lipids.

The results from the own experiment showing that 18:3 in MGDG is essentially controlled by the genotype of the maternal plant whereas 18:3 in TG is ruled by a plastome/genome interaction is consistent with the physiological background of 18:3 synthesis.

In leaves, de-novo fatty acid synthesis for polar lipids is attributed to the chloroplasts (Roughan et al. 1979, Drapier et al. 1982). A circular route was proposed because the major

compound of the desaturation pathway, 18:1, was mainly esterified to microsomal PC (Stymne and Appelqvist 1978, Slack et al. 1979). Furthermore, desaturation of 18:1 to 18:2 occurred in the endoplasmic reticulum. The diacyldiglycerol (DAG) was transferred to the chloroplast envelope followed by galactolysation and 18:3 synthesis (Hawke and Stumpf 1980, Murphy and Stumpf 1980).

In seed cotyledons, 18:1 in PC served as substrate for desaturation (Dybing and Craig 1970, Slack et al. 1978). From labeling experiments it appeared that DAG and PC containing 18:1 should act as precursors for triacylglycerol moieties esterified with polyunsaturated fatty acids. Roughan and Slack (1982) emphasized that the flow of labeling through the acyl and glycerol moieties of PC in cotyledons synthesizing polyunsaturated TG is closely analogous to that observed in the developing leaf during the accumulation of polyunsaturated galactolipids. So, the above mentioned interaction between plastome and genome during 18:3 synthesis might be elucidated on a molecular basis that is a common precursor, 18:1 PC, for 18:3 in both chloroplasts and the storage lipids. A significant correlation between 18:3 contents in galactolipids and triacylglycerol (Diepenbrock 1984) is in line with this hypothesis.

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