

POLYUNSATURATION IN THE SEEDOIL OF RAPESEED NOT  
INFLUENCED BY GRAFT-TRANSMISSIBLE FACTORS

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The establishment of rapeseed genotypes with an improved composition of their unsaturated fatty acids, i.e. a higher percentage of linoleic (C18:2 > 30 %) and a lower percentage of linolenic (C18:3 < 3 %) acid is a complicated and time consuming task. Due to strong environmental and marked maternal influences, only low correlations have been found between the contents of polyenoic fatty acids determined in half-seeds and in their progenies (Pleines 1988). One most important factor influencing the biogenesis of the unsaturated fatty acids is the temperature prevailing during seed development. Also maternal effects have been reported in rapeseed (Kondra and Stefansson 1970). Such maternal influences on seedoil fatty acid composition are attributable either to (1) cytoplasmic effects, to (2) the maternal seed tissue or to (3) translocation to the developing seed of fatty acids per se or transmissible regulatory factors derived from the maternal plant.

Exclusion of cytoplasmic, i. e. organell effects on the polyenoic fatty acid composition of the seedoil has been possible using reciprocal crosses (Pleines and Friedt 1989). Diepenbrock and Wilson (1987) have explained the maternal influence by nuclear and cytoplasmic gene interaction. They have assumed two different genetic mechanisms to control linolenic acid synthesis in the seeds; i.e. C18:3 synthesis via phospholipids at the endoplasmatic reticulum on the one hand, and origin from cytoplasmatically inherited monogalactosyl-diacylglycerol (MGDG) on the other hand. Maternal effects could be attributable to involvement of each of the two pathways in the storage lipid biosynthesis. But the linolenic acid content of the galactolipids amounts to a total of less than 1 % of that of the neutral lipids; moreover, C18:3 in the triacylglycerols derived from the plastidically inherited MGDG should be detectable in reciprocal crosses and, therefore, represent a cytoplasmic effect.

The maternal seed coat as well as the aleuron of rapeseed contain 6 % to 8 % oil at maturity and the triploid endosperm is degenerated already prior to the main period of seedoil synthesis. Moreover, no transport of fatty acids has been reported in plants. However, the contents of polyenoic fatty acids in the rape seedoil might well be influenced or controlled by transmissible factors.

MATERIALS AND METHODSMaterials

Two genotypes of spring rapeseed, Brassica napus L., were used, i.e. the conventional 'zero erucic' cultivar 'Duplo' and a mutant low in linolenic acid content of its seedoil (see Brunklaus and Röbbelen 1987). Doubled-haploid (DH) lines were produced via microspore culture. Each one DH line was chosen; from 'Duplo' (D) and the mutant (M) to produce the parent plants in the greenhouse at average temperatures of 18°C day and 12°C night.

Grafting experiment

Plants from M and D were taken for grafting at the developmental stage 57 (according to Schütte et al. 1982), i.e. the beginning of inflorescence enlargement. Rootstocks were prepared by decapitating the main inflorescence and making a transversal cleft of 1 cm in the remaining stem base. A wedge of corresponding length was cut in the scion just below the seventh (or eighth) node. The scion, then was inserted into the receptive stock and the two were tightly joined by wrapping with parafilm at the graft union. To avoid desiccation, grafted plants were covered with a moistened plastic bag and were transferred for four weeks into a growth chamber with a constant temperature of 12°C. Grafting occurred in a reciprocal manner. Seeds developed and matured during May and June in the greenhouse at mean temperatures of 23°C day and 15°C night. Selfed plants grown under the same conditions were used as a control.

Fatty acid composition of seedoils was determined from samples of 200 mg seeds using GLC analysis (Thies 1971). Concentrations of single fatty acids [palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids] were given in percent of the total fatty acids contents. The oleic acid desaturation ratio (ODR) was calculated as  $ODR = (C18:2 + C18:3) / (C18:1 + C18:2 + C18:3)$  and the linoleic acid desaturation ratio (LDR) as  $LDR = C18:3 / (C18:2 + C18:3)$ .

RESULTS

Fatty acid composition of selfed seeds from the two ungrafted parental genotypes and the reciprocal grafts are presented in Table 1. D and M differed particularly in their contents of polyenoic fatty acids (C18:2, C18:3) as reflected by the LDR value, although single fatty acids varied within each genotype. Such differences within genotypes reflect environmental effects which ranged from nearly 7 % (C18:1) to 1 % (C16:0) with given fatty acids.

Composition of seedoils derived from the reciprocal grafts clearly revealed the same differences as found in the selfed seeds from ungrafted plants. Seeds from the scions and rootstocks in both graft combinations exhibited more or less the same percentage of palmitic acid; but pronounced effects of the genotypes were reflected in the proportion of C18:2 and C18:3. The mutant character with a low linoleic acid and

correspondingly an increased linoleic acid content was similarly expressed in the rootstock and in the scion. M produced 3 % C18:3 and 25,8 % C18:2 in the rootstock and equal amounts in the scion ( 2,9 % C18:3 and 25,2 % C18:2). The same observation held true for 'Duplo'. This means that the genotype of the rootstock did not influence the fatty acid composition of the scion.

#### DISCUSSION

The published data of maternal effects on unsaturated fatty acid composition in rapeseed cover a range from complete (Bartkowiak-Broda and Krzymanski 1983) to low maternal influence (Rakow 1973) indicating genotypical differences.

Wang et al. (1989) analysing the fatty acid composition of individual lipids in several soybean tissues detected corresponding C18:3 patterns in the triacylglycerols of roots and seeds. They assumed that seeds may share common mechanisms for linolenic acid formation with roots, which, however, differ from those acting in leaves and stems. With the same species, Carver et al. (1987) in grafting experiments demonstrated the fatty acid composition of their genotypes to be determined by the maternal plant. They concluded that graft-transmissible factors originating in the leaf tissue may control the phenotypic expression of fatty acid percentage in the seed.

In rapeseed, Pleines and Friedt (1989), in particular under a warm temperature regime demonstrated complete maternal control of linolenic acid content in crosses having the low linolenic acid genotype as the seed parent. They assumed an interaction between the maternal genotype and nuclear genes of the embryo by transmissible metabolites regulating fatty acid desaturation in the seed.

Opposite to these results, we could not detect any maternal effect in our grafting experiment. The genotype of the scion did differ distinctively from the stock genotype in polyenoic fatty acid content of the seeds and particularly in the LDR (0,29 for D and 0,10 for M). But in each case, fatty acid percentage fully corresponded between the grafted individuals and the respective ungrafted plants. Therefore, in our rapeseed genotypes a transmissible factor produced by the root or by other stock tissues and affecting the polyunsaturated fatty acid composition of the seedoils could not be detected. We cannot exclude, however, transmissible metabolites produced in the pods or any other near-by vegetative tissue as a cause for maternal effects.

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Table 1. Means and ranges of individual fatty acids contents (%) of seeds harvested from ungrafted parents as well as rootstock and scion of reciprocal grafts between cv. 'Duplo' (D) and the mutant (M), respectively

Origin of seeds	Geno- type	n	Fatty acid					ODR	LDR
			C16:0	C18:1	C18:2	C18:3			
ungrafted	D	5	4,5	64,4	17,6	9,0	0,29	0,34	
			4,2-5,1	61,1-67,8	15,1-19,7	7,7-10,2			
ungrafted	M	4	4,8	60,1	27,3	3,3	0,34	0,11	
			4,5-5,3	56,2-63,6	24,6-29,8	2,7-4,3			
rootstock	D	10	4,4	66,0	17,3	7,2	0,27	0,29	
			4,0-5,2	49,0-71,3	14,4-30,0	5,9-9,1			
scion	M	10	4,5	62,7	25,2	2,9	0,31	0,10	
			4,1-5,3	56,5-67,2	22,1-29,5	2,2-4,0			
rootstock	M	9	4,7	61,9	25,8	3,0	0,32	0,10	
			4,0-5,1	58,3-69,0	20,6-29,1	2,3-3,9			
scion	D	9	4,9	65,6	17,3	7,0	0,27	0,29	
			4,1-7,3	53,1-70,7	13,8-26,4	6,1-8,1			