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Genetic control of linolenic acid concentration in seed oil of rapeseed (*Brassica napus* L.)

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Summary. Results from a diallel mating of two rapeseed lines with distinctly different linolenic acid concentration show that this trait is mainly under control of nuclear genes of the embryo. However, significant differences in reciprocal F_1, BC_1, and BC_2 indicate maternal control, which is realized by interaction between maternal genotype and nuclear genes of the embryo. Additionally, temperature exerts considerable influence on the degree of maternal control. Since no reciprocal differences are detectable in F_2, cytoplasmic factors seem not to be involved in the inheritance of linolenic acid concentration. Hypotheses on the physiological nature of maternal control of this trait are discussed.

Key words: *Brassica napus* – Rapeseed – Fatty acid composition – Linolenic acid

Introduction

Brassica species like *Brassica campestris*, *Brassica napus*, and *Brassica juncea* constitute the world's third most important source of vegetable oil at present (Anonymous 1988). Regarding the oil quality of *B. napus* (hereafter referred to as rapeseed), significant improvement has been achieved within the last decades by shifting commercial cultivation from traditional rapeseed, which is characterized by high concentrations of erucic acid (C22:1), to zero-erucic varieties. However, the nutritional quality of the oil can still be improved by increasing the levels of the dietary essential linoleic acid (C18:2) and decreasing the levels of α-linolenic acid (C18:3, cf. Beare-Rogers 1988). High concentrations of the latter fatty acid promote oxidation processes (Galliard 1980) which negatively affect the shelf life of oil and secondary products thereof.

Besides the elimination of erucic acid, improvements of the C18 fatty acid composition were achieved in the past by inducing high linoleic/low linolenic acid genotypes via chemical mutagenesis (Röbbelen and Nitsch 1975) and, more recently, by interspecific hybridization between these mutants and *B. juncea* (Roy and Tarr 1987). Since the phenotypic expression of C18 fatty acids is significantly influenced by genotype and environment, particularly by temperature during seed development (Trémolières et al. 1982; Pleines and Friedt 1988), it has been the objective of this study to investigate the mode of inheritance of linolenic acid concentration in a high × low cross under controlled temperature regimes in a phytotron.

Materials and methods

Two spring rape lines with entirely different linolenic acid concentrations in seed oil were crossed in a diallel set. The low linolenic acid line (LLA line) was derived from a complex cross as described above (Roy and Tarr 1987); seeds were kindly supplied by Dr. N. N. Roy, Australia. The high linolenic acid line (HLA line) was selected after three inbred generations in a breeding program for modified C18 fatty acid composition (Pleines 1988; Pleines and Friedt 1988).

Parental lines and reciprocal F_1 combinations were produced in a greenhouse at Gießen (FRG) during winter at average temperatures of 18 °C (day) and 12 °C (night) with additional light (18 h/day). The phytotron experiment at Rauischholzhausen/Gießen comprised two temperature regimes, under which parental lines and reciprocal F_1 were tested in four and seven replications, respectively. One replication consisted of four half-seed plantlets potted in a Mitscherlich vessel. All plants were grown under outdoor conditions until 1 week before beginning of flowering. From that date on up to maturity, plants were kept under two different temperature regimes in climate chambers.

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The day/night temperatures were set constant to 24.5°/18.0 °C in the 'warm' and to 14.5°/8.0 °C in the 'cold' treatment. Plants were harvested at complete seed maturity (stage 90 on BBA developmental scale; Schütte et al. 1982), i.e. after 68 days in the warm or 98 days in the cold environment. Relative humidity was set constant to 70% in both climate chambers. Artificial light was provided by sodium high pressure lamps with photosynthetically active radiation of ca. 200 $\mu M\ m^{-2}\ s^{-1}$ for 19 h per day.

At the beginning of flowering, parental and F_1 plants were isolated with gauze bags to obtain parental and F_2 seeds. At the end of full flowering (stage 65; Schütte et al. 1982), all isolations were removed in order to minimize microclimatical influences on the fatty acid pattern in developing seeds; rarely occurring new flowers were detached. F_1 and backcross seeds were produced with four replications per cross in both environments.

Sample size of parental and F_2 was 3 g of seeds harvested at random from all plants of each vessel. Fatty acid composition of F_1, BC_1, and BC_2 was determined on samples consisting of 15 single seeds; all samples were analyzed with two replications. Subsequent to homogenization of seeds, total lipids were extracted in petroleum benzine, solvent evaporated and thereafter transesterified in Na-methylate solution. Separation of transmethylated fatty acids was carried out by GLC on Chromosorb W-AW-DMCS coated with 6% BDS, as described by Thies (1971), with modified sample injection (Marquard 1987).

Single fatty acid concentration is given in percent of total fatty acids. Weighted midparental values were calculated for each cross and environment according to the following formula:

(%C18 fatty acid of HLA parent
·% nuclear HLA genes in hybrid
+ %C18 fatty acid of LLA parent
·% nuclear LLA genes in hybrid)/100.

Desaturation ratios were calculated as follows:

ODR (oleic desaturation ratio)
$= (\%C18:2 + \%C18:3)/(\%C18:1 + \%C18:2 + \%C18:3) \cdot 100.$

LDR (linoleic desaturation ratio)
$= \%C18:3/(\%C18:2 + \%C18:3) \cdot 100.$

The magnitude of desaturation ratios represents the amount of substrate which is successively desaturated from C18:1 to C18:2 and C18:3, thus providing a proportional measure of the activity of the respective desaturating enzyme(s) during seed development (Chérif et al. 1975).

Results

Means and variances of C18 fatty acids from the first mating set grown under greenhouse conditions are given in Table 1. The low linolenic parent (designated P_1) and the high linolenic parent (designated P_2) show overlapping variation for oleic and linoleic acid concentration, but entirely distinct linolenic acid concentrations. F_1 seeds grown on the LLA parent show marked prevalence for low linolenic acid concentration. On the other hand, the reciprocal F_1 does not differ significantly from the midparent value. Thus, the LLA × HLA cross indicates either maternal or cytoplasmic control of linolenic acid concentration, whereas the reciprocal combination indicates additive action of nuclear genes (alleles) of the embryo.

The linolenic acid concentrations of parents and reciprocal F_1, F_2, BC_1, and BC_2 combinations of the same cross were further tested under a cold (Fig. 1) and a warm

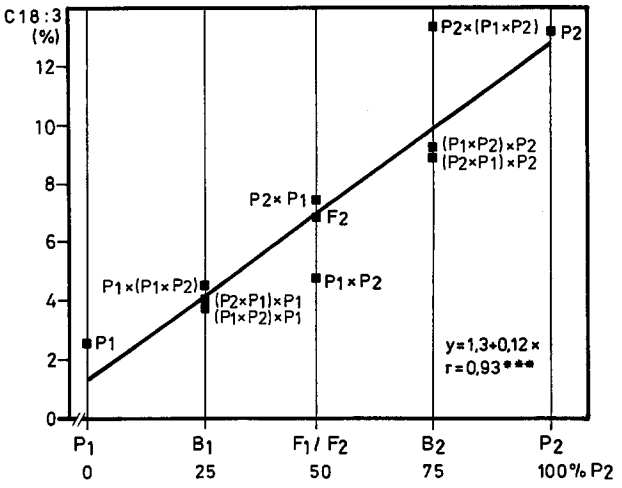

Fig. 1. Phenotypic expression of linolenic acid concentration in a diallel cross under a cold temperature regime, i.e. 13.1 °C average daily temperature (% P_2 = relative contribution of P_2 genes to the respective genotypes)

Table 1. Mean and variation of C18 fatty acid composition for parents and F_1 from a cross between high × low linolenic acid rapeseed under greenhouse conditions

Generation		C18 fatty acid pattern in % of total fatty acids								
		Oleic			Linoleic			Linolenic		
	n	Min.	x̄	Max.	Min.	x̄	Max.	Min.	x̄	Max.
P_1	25	55.1	61.1	67.1	23.7	30.0	36.5	2.2	2.9	3.9
$P_1 \times P_2$	30	52.3	60.9	70.7	18.6	27.2	33.5	3.2	4.6	6.7
Midparent			59.4			27.5			7.2	
$P_2 \times P_1$	26	53.6	56.8	63.7	24.2	29.3	32.6	5.3	7.1	9.2
P_2	25	53.9	57.6	60.8	21.8	24.9	27.5	10.2	11.5	12.5

Table 2. C16 and C18 fatty acid concentrations and desaturation ratios in a high × low linolenic acid cross under a 'cold' temperature regime in phytotron. ODR, LDR = oleic and linoleic desaturation ratios; midparent = weighted midparental value according to parental contribution to nuclear genes of the embryo; a, b, c = means with different letters differ significantly within corresponding generations (Student-Newman-Keuls test, $\alpha < 0.05$)

Generation	Cross combination		% P_2 nuclear genes	Fatty acid concentrations in %				Desaturation ratios	
	female	male		16:0	18:1	18:2	18:3	ODR	LDR
P_1	P_1	P_1	0	3.0	60.2	33.2	2.2	37.0	6.2
B_1	P_1	$P_1 \times P_2$	25	3.3	63.4a	28.4a	4.5a	34.1a	13.4a
B_1	$P_1 \times P_2$	P_1	25	3.5	59.5a	32.4b	3.9a	37.9a	10.5a
B_1	$P_2 \times P_1$	P_1	25	3.1	62.0a	30.4ab	4.0a	35.6a	11.6a
B_1	Midparent		25	2.9	60.4a	30.4ab	4.9a	36.9a	14.1a
F_1	P_1	P_2	50	3.1	67.0a	24.4a	4.8a	30.4a	16.5a
F_1	P_2	P_1	50	3.3	56.8b	32.0b	7.5b	41.0b	19.0b
F_1	Midparent		50	2.9	60.6c	27.5c	7.7b	36.7c	21.9c
F_2	$P_1 \times P_2$	$P_1 \times P_2$	50	2.9	63.5b	25.3a	6.9a	33.7a	21.5a
F_2	$P_2 \times P_1$	$P_2 \times P_1$	50	2.7	64.1b	25.1a	6.9a	33.3a	21.5a
F_2	Midparent		50	2.9	60.6a	27.5b	7.7a	36.7b	21.9a
B_2	P_2	$P_1 \times P_2$	75	3.2	54.6a	28.1a	13.3a	43.1a	32.0a
B_2	$P_2 \times P_1$	P_2	75	2.7	63.9b	24.1b	8.9b	34.0b	27.0b
B_2	$P_1 \times P_2$	P_2	75	3.0	61.8b	25.2b	9.2b	35.7b	26.7b
B_2	Midparent		75	2.8	60.8b	24.7b	10.4b	36.6b	29.8c
P_2	P_2	P_2	100	2.7	61.0	21.8	13.1	36.4	37.6

Table 3. C16 and C18 fatty acid concentrations and desaturation ratios in a high × low linolenic acid cross under a 'warm' temperature regime in phytotron. ODR, LDR = oleic and linoleic desaturation ratios; midparent = weighted midparental value according to parental contribution to nuclear genes of the embryo; a, b, c, d = means with different letters differ significantly within corresponding generations (Student-Newman-Keuls test, $\alpha < 0.05$)

Generation	Cross combination		% P_2 nuclear genes	Fatty acid concentrations in %				Desaturation ratios	
	female	male		16:0	18:1	18:2	18:3	ODR	LDR
P_1	P_1	P_1	0	3.1	70.2	23.4	2.1	26.6	8.2
B_1	P_1	$P_1 \times P_2$	25	3.9	78.8a	14.3a	2.1a	17.3a	13.1a
B_1	$P_1 \times P_2$	P_1	25	4.3	62.3b	29.7b	2.9ab	34.3b	8.8b
B_1	$P_2 \times P_1$	P_1	25	4.7	53.3c	37.1c	4.2b	43.8c	10.2ab
B_1	Midparent		25	3.1	69.2d	23.1d	3.4c	27.7d	12.4a
F_1	P_1	P_2	50	3.7	79.5a	13.7a	2.1a	16.6a	13.5a
F_1	P_2	P_1	50	4.0	60.6b	29.2b	5.2b	36.2b	15.1ab
F_1	Midparent		50	3.1	68.2c	22.8c	4.8c	28.8c	16.7b
F_2	$P_1 \times P_2$	$P_1 \times P_2$	50	2.8	65.8a	26.4a	3.9a	31.5a	12.7a
F_2	$P_2 \times P_1$	$P_2 \times P_1$	50	2.8	67.8a	24.5a	3.9a	29.5a	13.7a
F_2	Midparent		50	3.1	68.2a	22.8a	4.8a	28.8a	16.7b
B_2	P_2	$P_1 \times P_2$	75	4.5	59.8a	27.4a	7.6a	36.9a	21.6a
B_2	$P_2 \times P_1$	P_2	75	4.2	64.0ab	25.2ab	5.5b	32.4ab	17.8b
B_2	$P_1 \times P_2$	P_2	75	4.2	61.9a	26.8a	6.1b	34.7ab	18.7b
B_2	Midparent		75	3.1	67.2b	22.5b	6.1ab	29.8b	20.9b
P_2	P_2	P_2	100	3.1	66.2	22.2	7.4	30.9	25.1

temperature regime (Fig. 2). Linear regression analysis ($y = a + b\,x$), designating x as 0%, 25%, 50%, 75%, and 100% contributions, respectively, of HLA genes to the genotype of the embryo and y as the linolenic acid concentration of the respective cross, reveals distinct control of nuclear genes of the embryo. Coefficients of correlation amount to $r = 0.84^{***}$ in the warm and $r = 0.93^{***}$ in the cold environment (both significant at $\alpha < 0.001$), thus indicating predominant control of linolenic acid concentration by nuclear genes of the embryo. The 'determina-

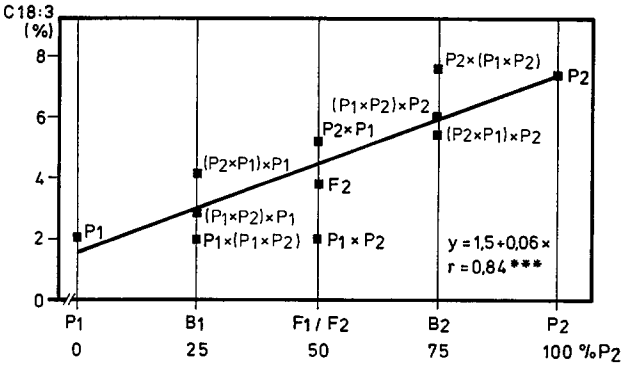

Fig. 2. Phenotypic expression of linolenic acid concentration in a diallel cross under a warm temperature regime, i.e. 23.1 °C average daily temperature (% P_2 = relative contribution of P_2 genes to the respective genotypes)

tion values' (r^2%) computed from the above-mentioned correlations show that the extent of nuclear gene control varies with temperature. In the cold environment, 86% of the linolenic acid variation can be attributed to nuclear gene action, in comparison to 71% in the warm environment. Temperature causes a considerably smoother slope in the warm ($b = 0.06$) as compared to the cold environment ($b = 0.12$).

Results of F_1 resemble those of the first mating (Table 1), except that linolenic content of the HLA × LLA cross exceeds the midparental value in the warm variant (Table 3). Contrary to F_1, the linolenic acid concentrations of reciprocal F_2 matings are neither significantly different from each other nor from the weighted midparent value in both temperature regimes (Table 2 and 3).

Discussion

The above observations confirm earlier results in rapeseed that linolenic acid concentration is to a certain degree covered by the genotype of the maternal plant (Thomas and Kondra 1973; Bartkowiak-Broda and Krzymanski 1983). However, contrary to the findings of Diepenbrock and Wilson (1987), cytoplasmic control of linolenic acid concentration of seed oil is not evident in our material. Inconsistent findings on the degree of maternal control were also reported from various crosses of soybean (*Glycine max*), where either complete maternal control (Martin et al. 1982) or additive inheritance (Wilcox 1985; Erickson et al. 1988) of linolenic acid concentration was detected in F_1.

From the results presented here, it can be concluded that temperature substantially modifies the degree of maternal control. In the warm temperature variant, nuclear gene effects of the embryo are completely covered by maternal effects in those crosses having the LLA line as seed parent. However, contribution of nuclear genes is still evident in respective crosses grown under cold conditions. In addition, maternal genotypes exert significantly different degrees of maternal control, i.e. maternal effect of the LLA parent is notably stronger than that of the HLA line. In BC_2, maternal control of the HLA parent in combination with 75% of HLA nuclear genes cover the remaining 25% of nuclear LLA genes under both temperature regimes.

In conclusion, it seems that the phenotypically measurable degree of maternal control is realized by interaction between the genotype of the maternal plant and nuclear genes of the embryo. Higher temperature modifies the degree of maternal control. Therefore, the nuclear genotype of an individual cannot be immediately deduced from its phenotypic character expression (e.g. C18:3 content), so that selection is complicated by the necessity of progeny tests. By way of contrast, pure embryogenic control would simplify and help accelerate breeding for low linolenic acid concentration, since segregation of cross progenies could already be evaluated in single seeds produced on F_1 plants, e.g. by means of the half-seed technique.

Since linolenic acid concentration in the seed oil is not cytoplasmatically inherited and no transmission of fatty acid molecules from the leaf or pod, i.e. maternal tissue, to seeds has been reported yet, other physiological causes for maternal control of this trait have to be assumed. One possible cause could be the synthesis of fatty acids in the seed coat, the aleurone, or endosperm layers. However, fat content of the above tissues amounts to less than 6% of the total fat content of mature seeds (Appelqvist 1972). Nevertheless, lower 1,000 kernel weights at high temperatures might increase that ratio and modify the fatty acid pattern of whole seeds to a greater extent.

Translocation of substantial amounts of linolenic acid from plastids to the cytosol could cause maternal control as well. True evidence for such a release has not been given so far, but indications for a possible exchange were reported for fatty acid mutants in *Arabidopsis thaliana* (Browse et al. 1986). However, the total amount of plastidial linolenic acid amounts to less than 1% of that in the triacylglycerol fraction of rapeseed oil (Diepenbrock 1984; Norton and Harris 1975). Thus, plastidial linolenic acid cannot have substantial influence on the fatty acid composition of storage lipids.

Furthermore, maternal control of linolenic acid concentration may possibly originate from translocable metabolites, which regulate fatty acid desaturation in seeds, although they are synthesized in vegetative parts of the plant. Indications of such an interaction were reported from grafting experiments in soybean (Carver et al. 1987). Since reciprocal differences of F_1 are mainly caused by changed OD activities in our crosses (Table 2 and 3), those metabolites, if present in rapeseed, must have a major impact on oleic- rather than on linoleic-acid desaturase.

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