RESULTS OF RECURRENT SELECTION FOR MODIFIED POLYENOIC FATTY ACID COMPOSITION IN RAPESEED (*Brassica napus* L.)

Pleines, S., R. Marquard and W. Friedt
Institut für Pflanzenbau u. Pflanzenzüchtung I, Justus-Liebig-Universität, Ludwigstr. 23, 6300 Gießen, FR-Germany

Introduction

After the elimination of erucic acid (22:1), further changes in fatty acid composition of the seed oil of *Brassica napus* have been possible by means of plant breeding. C18:3/18:2 ratio could be drastically reduced by mutagenic treatment (RÖBBELEN & THIES, 1980) and interspecific crosses to *Brassica juncea* (ROY & TARR, 1986). Furthermore, rapeseed oil for food consumption could be improved by increasing 16:0 content (JÖNSSON & PERSSON, 1983). In the future it seems possible now, that the variation of rapeseed oil leads to different cultivars with specific applications.

Material and Methods

Plant material: Plants for half-seed selection were grown under field conditions. For the phytotron experiment twelve lines with modified C18-fatty acid composition and 00-quality were selected after three generations of inbreeding. Six were spring- and six were winter-types, respectively (PLEINES, 1987).

Methods: Total glucosinolate content was determined according to MØLLER et al. (1984), and determinations of protein content were carried out as described by MARQUARD (1987). Fatty acid composition was determined by gas-liquid chromatography according to THIES (1971) with modifications previously described by MARQUARD (1987). Analytical samples were taken as random seed samples (2g). The half-seed method was carried out according to THIES (1971). Single plants derived from reared half-seeds were transferred to isolation cages to avoid cross-pollination in the field. Heritability estimations ("operative heritability") were done as described by STRUBE (1967) based on experiments in five environments (4 environments in phytotron chambers + 1 variant under field conditions). The activity of the individual desaturation enzyme-systems could be measured according to the following formulae :

ODR (oleic desaturation ratio) = %18:2 + %18:3 / %18:1 + %18:2 + %18:3 · 100; LDR (linoleic desaturation ratio) = %18:3 / %18:2 + %18:3 · 100. The magnitude of the desaturation ratios represents the percentage of the substrate which has been desaturated following the sequence 18:1 to 18:2 to 18:3. It is a measure for the average activity of the respective desaturating enzyme during seed-maturation.

Phytotron-experiment: From emergence until the beginning of flowering the plants were grown in Mitscherlich-vessels (4 plants/vessel) under field conditions. At the beginning of anthesis plants were transferred concurrently to phytotron chambers, where temperature and photoperiod varied as follows; "warm"-variant: day/night temperature of 25/18°C at 12 and 19 hours photoperiod respectively; "cold"-variant: day/night temperature of 17/10°C at 12 and 19 hours photoperiod respectively. Due to the difference of daylength, the average daily temperature of the two variants was 14.2°C for the "cold"- and 22.2°C for the "warm"-variant.

Results and Discussion

Results of a single half-seed selection in spring rape will be presented here. The regression coefficients of relative single fatty acid contents and desaturase activities in half-seeds *versus* random seed samples of their progeny were calculated in order to examine the efficiency of the half-seed selection. The regression coefficient for LDR was considerably high with LDR (single plant) = 8.2 + 0.70 · LDR (half-seed),(n=118, $p<0.001\%$), whereas the regression coefficients for all single fatty acid contents and for ODR were low and not significant. Despite a high variation of C18-fatty acids among the parental half-seeds only a small number of their progeny transmitted the modified fatty acid phenotype to the following generation. This findings are in accordance to other investigations with rapeseed (RAKOW & McGREGOR, 1973) and soybeans (WILCOX, 1985). In contrast to the benefit of the half-seed technique for the breeding of zero-erucic rapeseed, the method seems to be less effective for the selection of genotypes with specific C18-polyenoic fatty acid composition.

Besides a complex mechanism of inheritance involving influences of maternal sporophyte, embryo-genotype and cytoplasm (DIEPENBROCK & WILSON, 1987), the C18-fatty acid composition is considerably influenced by environmental factors. Therefore, influences of varying temperature and daylength upon fatty acid composition were studied in a

phytotron experiment, and the determination of the fatty acid composition was carried out on random seed samples. Analysis of variance revealed highly significant main effects of genotype and environment and highly significant interaction variances for single fatty acid contents and desaturase activities.

Fatty acid composition of the seed oil of all genotypes showed a distinct decrease in its degree of desaturation upon increasing temperature which is in accordance with other experiments (MARQUARD, 1980; TREMOLIERES et al., 1982). Due to the pronounced environmental variance, phytotron experiments appear to be well suited to uncover genotype X environment interactions concerning C18-fatty acids (Table 1). Environmental variance exceeded genotypic variance nearly fourfold for 18:3-content, whereas effects of environment on 18:2-content were smaller as compared to the total phenotypic variance. Though significant interactions were detectable, their magnitude was low in comparison to the main effects. Consequently, ranking of the genotypes according to different levels of single fatty acids did not differ greatly between environments. Therefore, estimated coefficients of heritability were comparatively high, as summarized for each fatty acid and for different desaturating systems in Table 2. Despite the unsatisfactory results concerning efficiency of half-seed selection, C18-fatty acid composition of random seed samples proved to be highly heritable. Heritability for ODR has been in the same range as for single fatty acids, while heritability of LDR was slightly lower. The h^2-values could be somewhat overestimated in this trial, because a separation of variance of years has not been possible. For corresponding estimations under different conditions, see BARTKOWIAK-BRODA & KRZYMANSKI (1983) and KONDRA & THOMAS (1975).

Tab. 1: VARIANCES OF FATTY ACIDS IN DIFFERENT ENVIRONMENTS

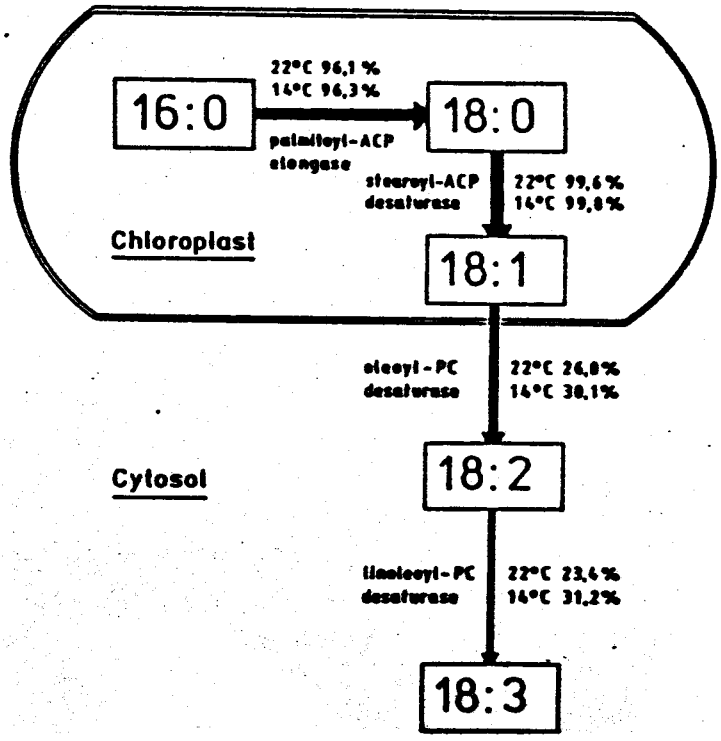
SOURCE OF VARIATION	DF	18:1	18:2	18:3	ODR	LDR
GENOTYPE (P)	11	273.1	140.8	31.3	288.9	138.8
ENVIRONMENT (U)	4	286.4	66.2	119.7	288.3	789.0
P X U	44	18.5	10.2	2.5	19.7	16.7
ERROR	180	1.8	1.1	0.3	1.9	2.3

Tab. 2: HERITABILITY COEFFICIENTS OF SINGLE FATTY ACIDS AND DESATURASE ACTIVITIES
($\sigma^2_G = \sigma^2_P - \sigma^2_{G/U}$; $h^2 = \sigma^2_G / \sigma^2_P$)

Palmitic	0.89
Oleic	0.93
Linoleic	0.93
Linolenic	0.92
Oleic-Desaturation-Ratio (ODR)	0.93
Linoleic-Desaturation-Ratio (LDR)	0.88

The influence of temperature on individual desaturation enzyme systems under two different daylength regimes is shown in Figure 1. Whereas the elongation and desaturation system located in the chloroplast are highly active and show only slight modifications upon changed post-flowering temperatures, the enzymes of the cytosol, such as oleoyl- and linoleoyl-PC desaturase were distinctly decreased in acti-

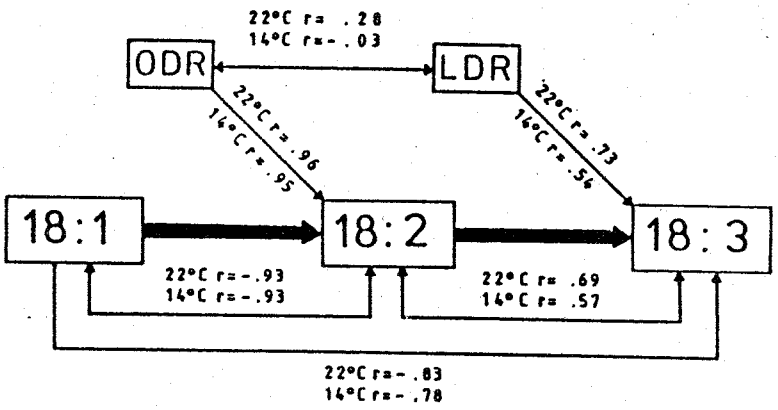
Fig. 1:



vity under "warm" conditions. An elevation of the average temperature from 14 to 22°C resulted in a decrease of OD-activity for 4.1% and of LD-activity for even 7.8%. Unfortunately, regulation mechanisms of the desaturating systems are complex and still not completely understood (FRENTZEN, 1986; MAZLIAK, 1979), which causes difficulties for the breeder (RÖBBELEN & THIES, 1980).

Correlations between fatty acids and desaturating enzyme activities at two different temperature regimes are shown in Figure 2. Negative correlations between oleic acid and the sbsequent desaturation products are pronounced with r = -0.93 between 18:1 and 18:2 at both temperature regimes and r = -0.83 at the "warm"- and r = -0.78 at the "cold"-variant between 18:1 and 18:3. A positive correlation of r =

Fig. 2: Correlations between C_{18}-polyenoic fatty acids and their desaturation enzymes



+0.63 is still observed between 18:2 and 18:3. Therefore, selection of genotypes with high 18:2 and low 18:3 content may be difficult. On the other hand, high 18:1 content at a simultaneously low 18:3 level is only feasible in genotypes with low OD-activity. Though OD- and LD-activities are not significantly correlated, OD-activity regulates 18:3-content indirectly *via* regulation of the amount of substrate (which means 18:2 here) for the subsequent desaturation step.

Two 00-spring-rape inbred lines tested in the phytotron showed the following variation regarding 18:1 and 18:3 contents in the seed oil over all environments: 73-79% for 18:1 and 4-8% for 18:3; ODR varied on a relatively low level between 17-25%. These genotypes could be useful for breeding rapeseed as an industrial crop with a single monoenoic fatty acid as main component in the seed oil.

Conclusions

1. The half-seed technique seems to be less effective for the selection of genotypes with specific C18-fatty acid composition than for the selection of low erucic acid contents in rapeseed.
2. Contents of C18-fatty acids and desaturase activities proved to be highly heritable (h^2 = 0.88-0.93) as estimated from random seed samples in phytotron experiments.
3. The average activity of 18:1- and 18:2-desaturase was calculated from the fatty acid composition of mature seeds. Both enzyme activities were not significantly correlated.
4. Due to the common influence of 18:1- and 18:2-desaturase on the 18:3 content, both enzyme activities should be considered in breeding for low 18:3 lines.

References

BARTKOWIAK-BRODA, I. & J. KRZYMANSKI (1983): Proc.Intern.Rapeseed Conf., Paris, 477-482.

DIEPENBROCK, W. & R.F. WILSON (1987): Crop Sci. 27, 75-77.

FRENTZEN, M. (1986): J.Plant Physiol. 124, 193-204.

JÖNSSON, R. & C. PERSSON (1983): Proc.Intern.Rapeseed Conf., Paris, 311-314.

KONDRA, Z.P. & P.M. THOMAS (1975): Can.J.Plant Sci. 55, 205-210.

MAZLIAK, P. (1979): IN: Low temperature stress in crop plants. (Eds.: LYONS, J.M. et al.), Academic Press, New York, 391-404.

MARQUARD, R. (1980): Habilitationsschrift Univ.Gießen.

MARQUARD, R. (1987): Fat Science Technology 89, 95-99.

MØLLER, P., A. PLÖGER, H. SØRENSEN (1984): IN: Adv.in the production and utilization of cruciferous crops with special emphasis to oilseed rape, Copenhagen, 11.-13.9.1984.

PLEINES, S. (1987): Diss.Univ.Gießen (in preparation).

RAKOW, G. & D.I. McGREGOR (1973): J.A.O.C.S. 50, 400-403.

RÖBBELEN, G. & W. THIES (1980): IN: Brassica crops and wild allies. Eds.: TSUNODA, S., K. HINATA, C. GOMEZ-CAMPO. Jap.Sci.Soc.Press, Tokyo, 253-283.

ROY, N.N. & A.W. TARR (1986): Z.Pflanzenzüchtg. 96, 218-223.

STRUBE, H. (1967): Diss. Univ.Hohenheim.

THIES, W. (1971): Z.Pflanzenzüchtg. 65, 181-202.

TREMOLIERES, A. & J.P. DUBACQ, D. DRAPIER (1982): Phytochemistry 21, 41-45.

WILCOX, J.R. (1985): Proc.World Soybean Research Conf. III. (Ed.: SHIBLES, R.), Westview-Press/Boulder, 380-386.